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RESEARCH · CONTROL · TEACHING

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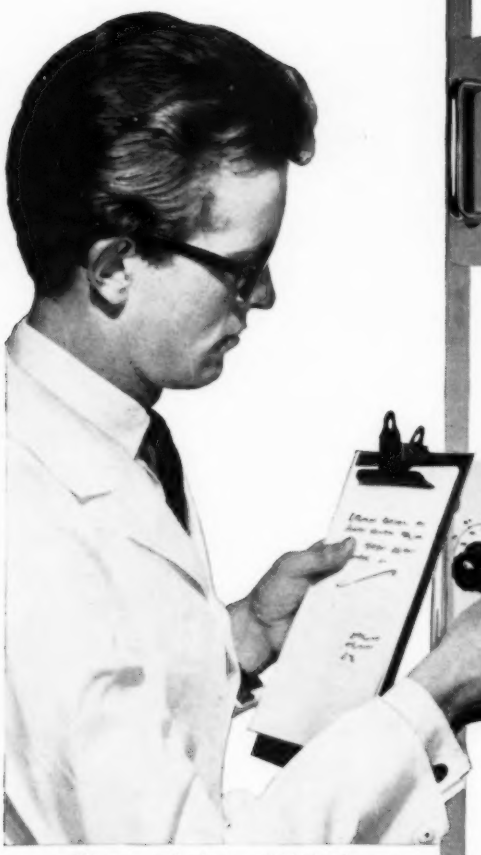

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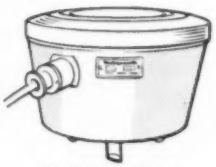

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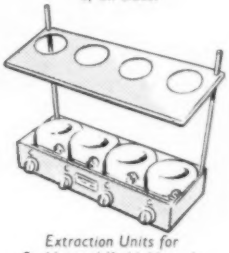

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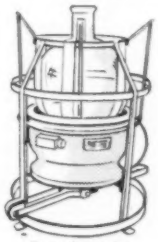

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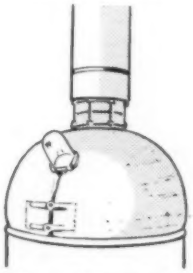

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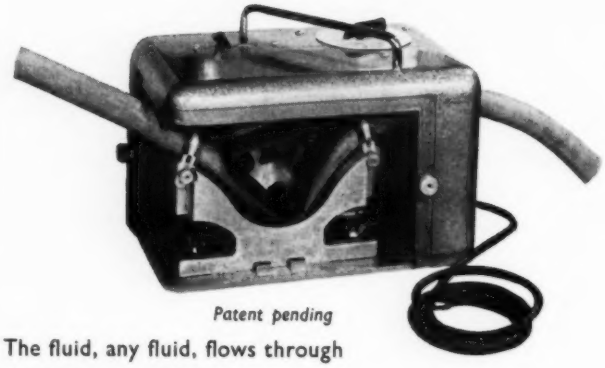

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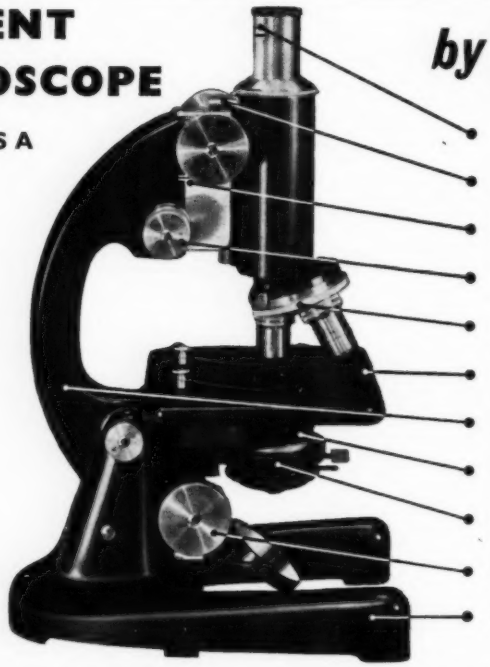

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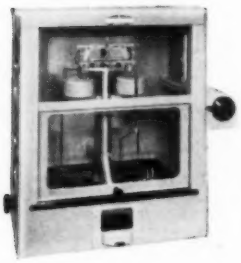

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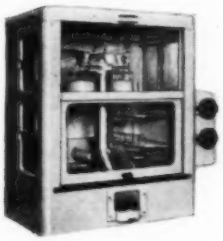

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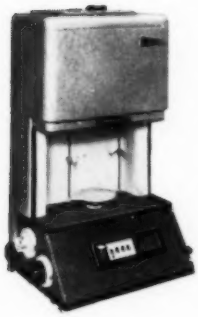

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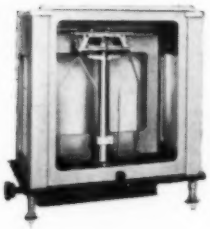

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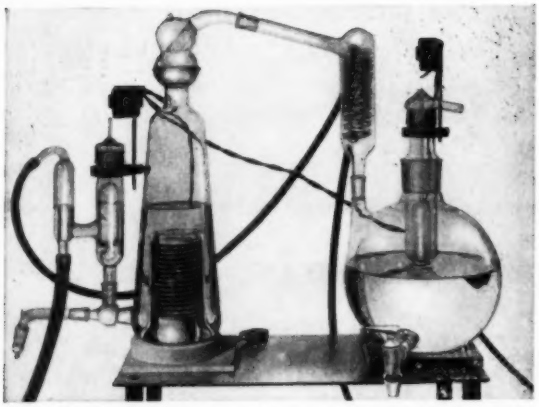

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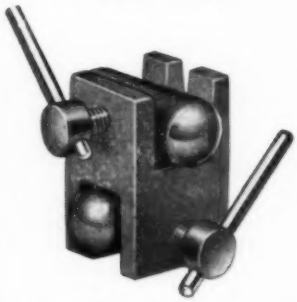

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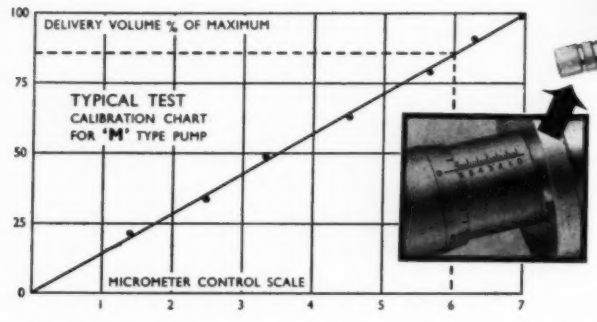

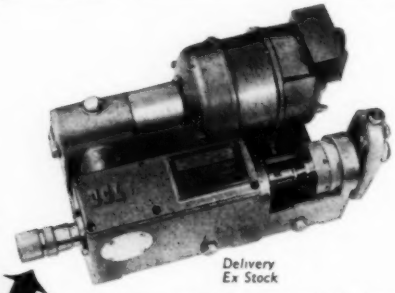

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EDITORIAL

NATIONAL PRINTING DISPUTE

Our printers are still faced with the problem of clearing a vast amount of work that has accumulated during the stoppage and, in order to assist them to make an early return to the normal publishing date, this issue of LABORATORY PRACTICE has had to be curtailed. We do assure our readers, however, that we anticipate a return to normal issues as from September next.

On Giving Papers to Scientific Societies

IN spite of the increased money spent on education today, and on young scientists in particular, it is still a common experience to listen to a paper given to a scientific society in a state of annoyance or boredom because the speaker has failed to pay attention to some very elementary but important points. It may be true that exponents of science, like politicians and preachers, are born rather than made, but a naturally poor speaker can become a reasonably fair one, for scientific papers at any rate, by paying a little attention to common failings in this type of work. A good research worker may feel that extra effort in this direction is unnecessary, but such an attitude is discourteous to his fellow-workers, and is merely a form of conceit. A considerable improvement can be effected by simply following the hints offered below.

The first thing is to decide what are the main points to be made in the paper, and to arrange the evidence for these in a clear and logical way. It is better to expound five points clearly than gabble through ten points and leave the audience in a confused state of mind.

Other things being equal, it is better to speak from notes than to read a manuscript, but it is better to read a manuscript than to muddle along, with frequent corrections and jumps forward and backward. If reading from a manuscript it is essential for the reader to hold his head up and speak clearly and slowly. Some people spend 15 minutes murmuring into their waistcoats as though only the front row had a right to hear

what the paper was about. The first time the young scientist gives a paper he should get a candid friend to sit in the back row and rehearse his paper to him. The timing of the paper should also be checked. Unless permission has been given, a paper should not last for longer than 15 minutes.

Nearly all scientific papers involve the showing of slides and it is amazing how even senior and experienced workers still make the most elementary errors in showing slides. Particularly bad is the habit of projecting slides containing a large number of figures. These are usually typed, and both figures and words are illegible from the fourth row and beyond. Slides of this character are bad enough, but it is unforgivable to put such a slide on and then, five seconds later when the audience has just had time to make out the headings to the columns, pass on to the next slide. 'These figures show clearly that . . .' chants the lecturer, when they do nothing of the sort. Slides are best drawn by hand, printing in black ink and having not more than six rows and six columns. Time should always be allowed for the audience to digest the data. These points have all been made time and time again, but speakers still blissfully ignore then. It is not only the lecturer who can be criticized. Some institutions are quite incapable of providing an efficient lantern in good working order, and an efficient operator. We have been present when the efforts of the lanternist have been more reminiscent of a music hall turn than of a scientific meeting.

To sum up, a carefully prepared paper, timed to last not more than 15 minutes, delivered clearly and illustrated by a few simple slides, will meet all the main requirements of a scientific communication. One or two jokes or witticisms timed to occur at 10 ± 1 minutes are not unwelcome, but too many will make the speaker suspect. A minute should always be allowed at the end for a brief summary. Even if the audience has not been with the lecturer all the time, they will usually be interested in hearing what he thinks he has proved.

We may offer one final word of advice—be careful in the use of well-worn phrases in your paper. A most amusing selection of these, complete with the most unkind interpretations, was recently published in '*The Link*', the house journal of Richardson Westgarth Limited. Some extracts from these are given in the Journal of the Institute of Biology for May this year, and a few are given below:

Introduction

'It has long been known that'
(I haven't bothered to look up the original reference.)

'Of great theoretical and practical importance'
(Interesting to me.)

Experimental Procedure

'Three of the samples were chosen for detailed study'
(The results on the others didn't make sense and were ignored.)

Results

'Typical results are shown'
(The best results are shown.)

'The most reliable values are those of Jones'
(He was a student of mine.)

Let him who reads papers run carefully!

British Space Research

The Prime Minister announced in the House of Commons on May 12 that a team of experts would be going to the U.S.A. to discuss Anglo-American co-operation in space research. The team has now been appointed by the Lord President of the Council and consists of:

Head of Team: Prof. H. S. W. Massey, F.R.S., Quain Professor of Physics at University College, London, Chairman of the British National Committee on Space Research, member of the Steering Group for Space Research and member of Executive Committee of COSPAR (Committee on Space Research appointed by the International Council of Scientific Unions).

Members: Dr. R. L. F. Boyd, Reader in Physics at University College, London. One aspect of his work is the study of the upper atmosphere and astrophysical problems.

Mr. J. Gait, Royal Aircraft Establishment, Ministry of Supply. In charge of the Skylark programme for upper atmosphere research by vertical sounding rockets.

Mr. R. N. Quirk, Secretary, Office of the Lord President of the Council.

Mr. M. O. Robins, attached to the Royal Society for work with Prof. Massey at University College, London, on space research activities. U.K. delegate to COSPAR.

Mr. G. D. Robinson, Deputy Director, Physical Research at the Meteorological Office.

Prof. J. Sayers, Professor Electron Physics, Birmingham University. One aspect of his work is the design of experiments and of the methods of processing information from space vehicles.

Dr. R. L. Smith-Rose, C.B.E., Director of Radio Research, Department of Scientific and Industrial Research.

The team will discuss United Kingdom space research proposals with United States National Aeronautics and Space Administration. The discussions will cover instrumentation of satellites and launching requirements as well as possible collaboration between United Kingdom, United States and other countries.

The Association of Consulting Scientists

At the recent Annual General Meeting of the Association of Consulting Scientists (the Chairman, Dr. J. G. Davis presiding) the Hon. Secretary gave a report on the provisional Committee's work including the preparation of a Constitution and Rules, which was debated. A report of the work to date of the Joint Committee with the Royal Institute of Chemistry was also presented.

It was stated that following the formation of the Association numerous felicitations and enquiries had been received, many from abroad.

The Chairman stated that the Provisional Committee recommended the appointment of a Council of nine members. This was accepted by the members present who thereupon elected the following Officers and Council of the Association for the ensuing year:

Chairman, Dr. J. G. Davis; *Hon. Treasurer*, Dr. G. W. Ferguson; *Hon. Secretary*, Mr. W. H. Stevens; *Other members of the Council*, Dr. M. Barent, Dr. H. H. Chambers, Dr. Julius Grant, Dr. J. H. Oliver, Mr. O. W. Roskill, Mr. F. G. Sarel Whitfield.

ORIGINAL CONTRIBUTION

THE ASSESSMENT OF HIGH REFLECTING FILMS FOR INTERFEROMETRY OR MONOCHROMATIC FILTERS

by S. Tolansky, F.R.S.

University of London

MULTIPLE-beam interference systems are extensively used for (*a*) Fabry-Perot spectroscopic interferometers, (*b*) microtopographical studies of surfaces and (*c*) narrow band-pass optical interference filters. For these it is necessary to employ high reflecting thin films and of these in effect three types have found employ. These are (1) silver films for Fabry-Perot interferometers designed to cover a wide spectral range, from the blue to the infra-red, (2) aluminium or aluminium-alloy films for Fabry-Perot instruments to be used over a wide spectral range, from the far blue down into the ultra-violet and (3) dielectric multilayers for very narrow spectral ranges in the visible region, either for Fabry-Perot interferometers, narrow band filters or microtopographical studies. (Ultra-violet multilayers have not yet been adequately exploited.)

Dielectric multilayers are often attractive because with them one can secure a very high reflecting coefficient, even 0·97, yet associated with a relatively low absorption coefficient, which can be slightly less than 0·01. Such a system has a very high light efficiency, yet the extremely narrow wavelength restrictions are such as still to leave an important place for well-made silver films. Again, we have demonstrated that the surface micro-contouring efficiency of a multilayer system is suspect, if such a multilayer is used to coat a complex topography under study; which is not the case with silver, which contours with astonishing fidelity. Thus it is clear that on numerous occasions a correctly deposited silver film is still often the best to use.

The best silver films are those deposited in a good vacuum by thermal evaporation and a good film should exhibit three characteristics. First it must have a high reflectivity, second the absorption should be low, and third it must contour faithfully. These conditions can be satisfied by a film of the correct thickness if rapidly deposited in a vacuum which is at least 10^{-5} mm. and if particular attention is placed to the cleanliness of the vacuum system and the surface receiving the deposit. Experience shows that both residual gas impurities and surface contaminants on the face to be coated can seriously affect the value of the absorption coefficient. Indeed we have experimentally established that there is little difficulty in securing a high reflectivity, the real problem is to obtain an adequate transmission with a high reflectivity and this is only possible if the absorption of the film is low, a matter which requires rigid cleanliness conditions.

An examination of the interferometric optical properties of thin films shows that the absorption is the critical factor which limits the film thickness that can be adopted and this in turn limits the reflectivity which can be reached. We have long ago established that the effect of the thin-film absorption on multiple-beam interference fringes is two-fold. Writing the fractions of the incident light reflected, absorbed and transmitted, respectively as R, A and T (clearly $R + A + T = 1$) then it can be shown that transmission fringes, by virtue of the absorption, are reduced in intensity by a factor $1\Big/\left(1+\frac{A}{T}\right)^2$. In the case of reflection fringes there is no effective light loss but on the contrary there is a loss in the fringe *visibility* by a factor also depending upon A/T. When absorption is high the fringes can barely be seen or photographed, although they still remain narrow.

High resolution demands as high a value of R as is consistent with as low a value of A as is possible. The optimum silver thickness is a critical matter, but it is stressed again that even this optimum thickness can be most seriously affected by an increase in absorption due to even slight impurity conditions. Although some workers have reported quite low absorptions for silver films (and we too have at times achieved this) the securing of very low absorption appears to be a matter of fortunate chance with techniques at their present state. It is our belief that mercury vapour pumps with liquid-air traps are probably superior to oil diffusion pumps in this respect of low absorption and it might be conjectured that with oil pumps a thin oil film creeps over the whole equipment, even on the surface receiving the silver. As the silver film may be perhaps 200 atom layers thick, it needs very little oil to contaminate this to produce an increase in the absorption coefficient.

TABLE I

Film	*Reflectivity R*	*Transmission T*	*Absorption A*
X	0·85	0·13	0·02
Y	0·90	0·07	0·03
Z	0·95	0·01	0·04

We find that under *average* working conditions (at times improved upon) it is possible to obtain the following figures for silver films, using green mercury light. The reflectivities, absorptions and transmissions depend upon the film thickness. In Table I are shown

data for three representative films, of increasing thickness, selected for increasing reflectivities. The data were obtained with a precision reflectometer.

It is seen that as the film thickness grows to increase the reflectivity, so also does the absorption increase. Usually the intensity of the fringe pattern drops catastrophically in transmission and the visibility suffers acutely in reflection if the reflecting coefficient is increased beyond 0·95. For in the case of film Z the intensity reduction factor $1/\left(1+\frac{A}{T}\right)^2$ equals 1/25th

In these regions a small increase in A has a very big effect. If the absorption is increased by the small amount of 0·005 this reduces T to 0·005 and the intensity reduction factor now becomes 1/100th, and all this decrease is due to a mere $\frac{1}{2}$ per cent increase in the absorbed light.

To assess the required film it is of course possible to set up a precision reflectometer to determine R and T, but in fact the determination of the small quantity T is a difficult matter. Such procedures are in practice to be avoided and we therefore employ a simple empirical device which we have found enables us to select admirable mirrors for interferometry. That the method of selection is sound is proved by the excellent performance of the mirrors chosen, for they give extremely sharp fringes with very high contrast, which is indeed the purpose of the selection. The method does not give values of R, A and T, it merely permits selection of mirrors which will, when used, give the required fringes.

This simple selection technique is as follows:
When the silver is deposited on to the surface under study, there are placed alongside of it, on either side, small strips of glass, of some 4 cm. in length. These are subjected to the identical cleaning treatment as the receiving surface and are simultaneously coated with the same deposit. On removal from the vacuum the two pieces of silvered glass are held close to the eye, held nearly parallel, but not quite. Through them a 'Pointolite' lamp is viewed at fairly close quarters (a bright filament lamp is an alternative, although not as good). By imposing a slight tilt between the two surfaces a train of successively multiple reflected images of the light source is seen. These fall off slowly in intensity and change colour progressively from a deep blue, then to yellow and ultimately almost to red. With really good silvered films a surprisingly large number of images can be counted. The number seen for any particular light source is determined by the values of R, T and A. Taking as unity the intensity of the light source, the intensity of the nth image is $In=T^2R^{2n}$. As this can also be written $In=(1-R-A)^2\,R^{2n}$ it is seen that the intensity of the nth image is a function only of R and A.

Let us make a comparison of the number of images which will be seen by the three films X, Y, Z for which data have previously been given. One finds in practice that with the particular light source advocated, an average good film, Z, shows easily no less than 60 clearly discernible images. The tail-end is a little difficult to decide, but this is of no significance. One mentally decides to stop at a given faint image and conveniently calls that the end of the train of images. (Important note. *The test should always be carried out in a darkened room.*) Substituting the experimental figures shows that the intensity of the 60th image is $0{\cdot}2\times10^{-6}$ that of the light source, i.e. one five millionth. This then is adopted in this instance as the threshold for image visibility.

Now the image intensities depend in a very critical way on R and A, especially when R approaches unity. A simple calculation shows that for the next film, Y, this same image intensity ($0{\cdot}2\times10^{-6}$) is reached already at the 46th image, as compared with the 60th image for film Z. Whilst for the film X, this intensity threshold is reached at the 34th image.

Clearly the respective image counts 60, 46 and 34 offer an immediate and rapid assessment of the combined values of R and A and this assessment is adequately sensitive. Our empirical rule, born of years of experience, is to accept as suitable a pair of mirrors which give a count of at least 60 images. It will always be found that such mirrors give fringes which are sharp, of adequate intensity and of good enough contrast.

Not infrequently, as many as 80 images can be counted, which can only come from a reflectivity better than 0·95 and an absorption less than 0·04. An 80 count leads to exceptionally good fringe definition. On a very few rare occasions (certainly less than one in a thousand) as many as 100 images have been counted. Fringe definition has then been superb, but in any case atmospheric attack quickly reduces the count to 80 or so.

After some familiarity, it is not even necessary to make a count. For when R is high there is a very slow fall-off in image intensity and with this is associated a characteristic slow colour change. The combination of slow fall-off and colour change often enables an expert assessment to be made at a glance. The colour change of the image is very distinctive. The first image is a characteristic deep whitish-blue. Successive images slowly move along the spectral range to a deep yellow and ultimately to an orange red. The reason, of course, is that the inherent reflectivity of silver is higher in the red than in the blue. Progressively the blue, with its lower reflectivity, is first lost, leaving a yellow image, and so on. Experience shows that the *slower* the approach to a deep yellow colour the better is the silver film. In other words the blue colour persists longer for a better film than for a poorer film. The colour appraisal is in every way as good as an image count, and much faster.

The image counting technique can be used equally for dielectric multilayers and for aluminium mirrors. In the case of the dielectrics only an image count can be used, for the image colour characteristics differ entirely from those which appear with silver. With the multilayer there is one narrow wavelength range for peak reflectivity, and reflectivity drops rapidly on either side

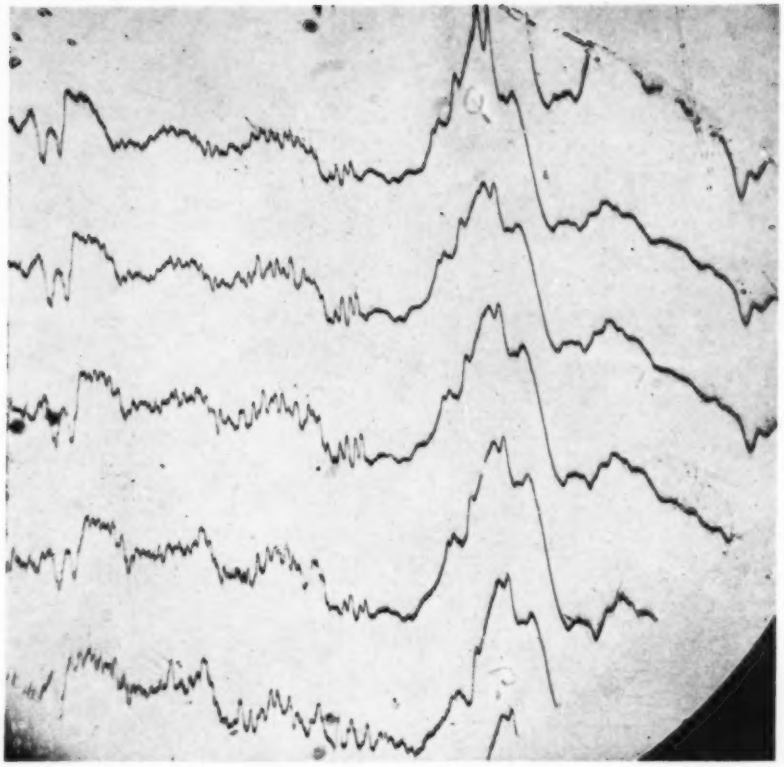

Plate 1.

of the peak if the multilayer is well made. The result is that all images after the first one or two have the same colour, which is in fact the peak wavelength colour. The only parameter available for assessing the reflectivity is then the count of images, which does however suffice to give an excellent measure for interferometric performance.

When the counting image technique is applied to aluminium mirrors their inferiority to silver in the visible region is immediately manifest. For aluminium reflectivity is of the order of 0·85 and absorption can approximate to 0·10. It is uncommon to be able to exceed a count of 25 images. Indeed 30 would usually appear to be an exceptionally good aluminium mirror for interferometry. The justification for aluminium is in the region below a wavelength of 4,000A, for below this, silver reflectivities fall rapidly down to the low value of some 0·04.

Plate 1 shows a multiple beam interferogram given by silver films with a count of 80. This is a x50 interferogram showing the state of polish on a diamond surface, polished for experimental purposes. The fringe definition is superb and the way in which the highly sharpened fringes contour the surface microtopography is beautifully rendered. The extreme fringe sharpness is evidence of a very high reflectivity. But these are *reflection* fringes. The very high contrast proves that the absorption in the film must be low. Clearly there has been no need for an elaborate reflectometric determination of R and A. The 80 count has been an adequate guide, indicating silverings which satisfy the most stringent interferometric requirements.

Rutherford Memorial Appointments

Under the terms of the scheme to commemorate the late Lord Rutherford of Nelson the following appointments have recently been made by the Council of the Royal Society:

Rutherford Memorial Lecture.—Sir Lawrence Bragg, O.B.E., F.R.S., has been appointed Rutherford Memorial Lecturer for 1960, to deliver the lecture in New Zealand.

Rutherford Scholarship.—Mr. D. J. Tritton, B.A., now working at the Cavendish Laboratory, Cambridge, has been appointed Rutherford Scholar for 1959 for three years, to investigate the relationship between the manner of production of turbulence in a fluid flow and the large eddy structure of that turbulence, at the department of aeronautical engineering, Indian Institute of Science, Bangalore.

ARTICLES

The Estimation of Physiologically Active, Naturally Occurring Substances in the Tissues and Body Fluids: Acetylcholine, L-Adrenaline, L-*nor*adrenaline, Histamine, Serotonin and Substance P.

by J. J. Lewis and N. G. Waton

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PART XI—SUBSTANCE P

IN 1931 Euler and Gaddum[214] prepared extracts from horse and rabbit brain and horse small intestine which caused a fall in blood pressure when injected into ether-anaesthetized, atropinized rabbits. The substance responsible for the depressor effect was established not to be histamine because histamine does not cause a fall in the rabbit's blood pressure. It was not acetylcholine because atropine abolishes the muscarinic actions of acetylcholine. The extract was called P and the active substance it contained became known as Substance P. Euler and Gaddum found that their preparation also stimulated the isolated, atropinized rabbit intestine.

Chemical Nature

Substance P appears to be a basic polypeptide or mixture of polypeptides. The impure substance is precipitated from solution by ammonium sulphate and other protein precipitants. It is readily dialysed, soluble in water in dilute acids and in ethanol and methanol, but insoluble in ether and chloroform. In dilute acid solutions (pH 3 to 6) substance P can be heated to 100°C. for 20 minutes or longer without loss of activity[215]. It is rapidly destroyed by boiling in a dilute solution of sodium hydroxide, by strong acids and alkalis, or by incubation with certain tissue extracts, trypsin or chymotrypsin. Substance P is amphoteric and has an isoelectric point at pH 6·4 to 7·0.

Biological Properties

Substance P stimulates preparations which contain plain muscle, for example the guinea pig ileum, hen uterus, hen rectal caecum, rabbit oesphagus, jejunum and stomach and the gall bladder of the guinea pig. It has apparently no action on bronchial smooth muscle. It increases the tone and spontaneous activity of isolated intestine and uterus and its stimulant effects are not abolished by atropine or antihistamines or ganglion blocking agents. Substance P causes vasodilatation with a fall in blood pressure in the atropinized rabbit and in the chloralosed cat and dog. In the cat anaesthetized with urethane a depressor effect may not be seen at all, or may be feeble.[218] Variable effects are also shown on the urethane-anaesthetized rabbit.[214]

Occurrence and Biological Function

Substance P is found together with histamine, 5-hydroxytryptamine, choline and acetylcholine in the wall of the gastro-intestinal tract. The highest concentrations are in the jejunum, duodenum, colon and rectum and the largest proportion is found in the muscular coat and muscularis mucosae. It is believed to be a local hormone concerned in the regulation of the movements of the gastro-intestinal tract and with folding and unfolding movements of the mucous membrane which go on in all parts, but especially in the stomach, intestine and colon. Bjurstedt and his colleagues[218] have noted similarities between substance P and cholecystokinin (a substance believed to act as a specific local hormone stimulating the gall bladder).[219] Both substances occur in extracts of intestine, cause contractions of the isolated, atropinized gall bladder of the guinea pig and of the isolated, atropinized rabbit intestine; both are destroyed by heating with alkali but cholecystokinin has no action on the rabbit's blood pressure. Euler[222,223] found a substance with properties similar to those of substance P in humen semen and extracts of the prostate glands of various animals. Bloch and Necheles[224] and Vogt[225] have observed that stimulation of the vagus causes the release of a substance P-like compound from the wall of the stomach which can be demonstrated in the gastric juice, and may be responsible for the atropine resistant effects of vagal stimulation in the gastro-intestinal tract which are seen in some species. Substance P has also been found in the retina and in the grey matter of the brain. The hypothalamus and basal ganglia are rich in substance P. Small amounts are found in peripheral nerves and peripheral ganglia. In Hirschsprung's disease, a condition in which a portion of the recto-sigmoid contains no ganglion cells in either Auerbach's plexus or Meissner's plexus (and, therefore, does not show peristalsis), it has been shown that there is a significantly lower concentration of substance P in the inactive region. This indicates that the substance P content and the presence of intramural ganglia are linked together.[227]

Identification of Substance P

1. The actions of substance P on smooth muscle are not antagonized by atropine or mepyramine, which abolish respectively contractions due to acetylcholine and histamine. When the extract is added to a bath containing Tyrode's solution to which atropine and mepyramine have been added in quantities sufficient to abolish contractions due to acetylcholine, histamine

and related compounds, there is a characteristic slow contraction. If the extract is then boiled for a few minutes with strong acid or strong alkali its activity is abolished. If doses of unboiled extract are given at short intervals (less than five minutes) tachyphylaxis is shown.

2. Extracts containing substance P cause a contraction of the atropinized rabbit ileum.

3. They cause a fall in arterial blood pressure when injected into atropinized rabbits under urethane anaesthesia. The atropinized rabbit does not give a depressor response to acetylcholine and in this species histamine has no depressor action.

Preparation of Extracts containing Substance P

In 1931 Gaddum and Euler[214] prepared extracts with substance P activity from horse small intestine. The mucous membrane was scraped off, the underlying muscle tissue minced and suspended in acid alcohol to give a suspension with a final concentration of from 72 to 80 per cent of alcohol and containing 1 ml. *N*-H_2SO_4 to every 100 ml. alcohol. The suspension was allowed to stand for an hour, stirred from time to time and then filtered. The filtrate was stored in a cold room overnight and the alcohol removed under reduced pressure. Fats were extracted by shaking out with ether, which does not dissolve substance P. The aqueous extract was used for experiments on the rabbit's blood pressure and isolated rabbit intestine, or was purified further (*see* original paper for description).

In 1935 Gaddum and Schild[216] prepared extracts from horse intestine by scraping off the mucous membrane, mincing the tissue which remained and putting the mince into an excess of acid 96 per cent ethanol which dissolves substance P and histamine. Aqueous trichloracetic acid can be used but it extracts adenosine compounds insoluble in concentrated ethanol. Three or four ml. of alcohol were used for each g. of tissue and 1 ml. 2*N*-H_2SO_4 added to the alcohol for every 60 g. of tissue. After standing for an hour the insoluble material was filtered off. The filtrate was concentrated by evaporation under reduced pressure, defatted by shaking with ether and finally evaporated under reduced pressure to small bulk.

In 1936 Euler[220] described a method for obtaining substance P from horse intestine. Horse small intestine is freed from fat and other extraneous tissues, split longitudinally and the contents removed. It is cut into pieces, weighed and boiled for ten minutes with an equal amount of water at pH 4. The extraction is repeated three times, the extract filtered and the filtrate concentrated by boiling at pH 4 until 1 ml. is equivalent to 5 to 10 g. of fresh tissue. The pH of the concentrate is adjusted to 3 by means of hydrochloric acid and ammonium sulphate added to half-saturation. The suspension is stored in the ice-box for about 24 hours to allow precipitation to be completed. The precipitate is collected, redissolved and purified by re-precipitation several times and finally collected and dried. The product is a brown powder with the characteristic action of substance P on the blood pressure of the atropinized rabbit and rabbit jejunum. This method of preparation eliminates contamination with histamine, choline esters and adenosine derivatives.

A method which extracts both substance P and histamine from the alimentary tract of dogs has been described by Douglas and his colleagues,[221] who employed it in the estimation of the distribution of histamine and substance P. The dogs are anaesthetized with chloralose and bled out; the entire alimentary tract is removed from the animal whilst it is still alive, cut into sections and until required for use is stored in saline in the ice-box.

The tissue is weighed, placed in a mortar, 2 ml. of *N*-HCl per g. of tissue added and ground with sand. Ten ml. of water per g. of tissue and a few ml. of saline are then added and grinding continued. The ground tissue and fluid extract are boiled for about one minute and filtered. The filtrate is neutralized with N-NaOH and tested on the atropinized guinea pig ileum for histamine and on the atropinized-mepyraminized guinea pig ileum for substance P.

Pernow[215,227] describes a method of extracting substance P which gives a high yield of an extract practically free from histamine, 5-hydroxytryptamine, choline derivatives and darmstoff. The washed tissue freed from fat and cut small is boiled for about ten minutes in distilled water which has been adjusted to pH 4 by addition of sulphuric acid. The acid extract is filtered, a second extraction is then made, the filtrates combined and concentrated *in vacuo*. To the concentrated extracts 2 volumes of 95 per cent ethanol are added and the extract stored in the refrigerator for 12 hours. This precipitates inert protein which is filtered off. The alcoholic extract is again concentrated *in vacuo* and 3 volumes of 95 per cent ethanol added to precipitate more inert protein which is removed by filtration and the filtrate concentrated once again. A substance P containing precipitate is obtained by adding ammonium sulphate to 70 per cent saturation. The precipitate is redissolved in water and re-precipitated with ammonium sulphate. This gives a very active preparation of substance P with very little contamination from other interfering substances.

Pernow[215,227] has also described a method of purifying the crude extract. The crude extract (prepared as described above) is dissolved in water. Methanol is added to the solution and a precipitate forms. This is filtered off and the filtrate passed down an aluminium oxide column. The substance P is adsorbed on to the column and can be eluted by means of a 50 per cent solution of methanol. The methanol extract is concentrated under reduced pressure and ethanol added to 80 volumes per cent. The ethanol-containing solution is passed down a second aluminium oxide column and eluted with 50 per cent ethanol. The 50 per cent ethanol extract, when evaporated to dryness under reduced pressure, gives a preparation containing about 500 units of substance P activity per mg. Further purifications can be carried out by partition chromatography on a

cellulose column, using as solvent a mixture of butanol, acetic acid and water. By this method Pernow obtained extracts which contained up to 3,000 units of activity per mg.

Biological Estimation

Rabbit Blood Pressure Method.—Gaddum and Schild[216] employed this method for the investigation of depressor substances obtained from horse small intestine. Rabbits are anaesthetized with ether and both vagi severed. The arterial blood pressure level is recorded in the usual way and drugs and extracts injected intravenously. Atropine is given to exclude effects due to acetylcholine and other choline esters. Gaddum and Schild used male Dutch rabbits which weighed from 1·0 to 1·5 kg. The usual anticoagulant fluid (a saturated solution of sodium sulphate) was replaced by an intravenous anticoagulant, using Ringer's solution in the cannula. They found a considerable variation in the sensitivity of rabbits to intestinal extracts.

Guinea Pig Ileum Method.—Pernow[217] has adopted the following method. A strip of guinea pig ileum is suspended in an organ bath containing 30 ml. of Tyrode's solution which contains glucose and is gassed with oxygen. The bath fluid contains 0·2 μg. of atropine sulphate and 1 μg. of the antihistamine Antergan. Addition of atropine and Antergan eliminates any effects due to acetylcholine or histamine.

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BOOK REVIEWS

Applied Microscopy and Photomicrography, by H. M. Malies, F.R.M.S. London: Fountain Press, 1959. 22s. 6d. pp. 143.

This is a very elementary introduction to the practice of microscopy. The book suits the technical assistant who has had little formal scientific training. It is fully illustrated with very clear diagrams, which are considerably simplified, with advantage for the type of reader aimed at.

This is essentially a practical handbook; indeed the only formula that appears is a bald statement of the definition of numerical aperture, with little real explanation. It is probable that any laboratory assistant who studies this handbook will improve his skill in manipulation of the microscope and will obtain better pictures, but he will not learn very much about the real principles behind the instrument. Phase contrast microscopy is described in a mere four pages. Interference microscopy is relegated to one paragraph plus reference to an instrument maker's catalogue.

The book suffers from a defect curiously common to elementary handbooks on the microscope, and this is the relative neglect of the metallurgical type of instrument for *opaque* objects. This whole subject is dismissed in four pages. Yet the geologist, petrologist, physicist, mineralogist and especially the metallurgist and the engineer study opaque objects. If a chapter can be devoted to the binocular microscope surely the opaque object deserves more space. One would have liked to have seen a chapter on the polarization microscope.—S. TOLANSKY.

Noise in Electron Devices, edited by L. D. Smullin and H. A. Haus London: Chapman and Hall, Ltd., New York: John Wiley and Sons Inc. 96s. pp. 413 + xvi.

This consists of a group of individual contributions to a summer course held at the M.I.T. on Noise. There are seven contributions as follows: (1) Shot noise from cathodes (C. F. Quate); (2) Low-frequency noise in vacuum tubes (A. van der Ziel); (3) Noise in electron beams (H. A. Haus); (4) Grid-control tube noise (T. E. Talpey); (5) Low-noise travelling-wave tubes (R. W. Peter); (6) Semi-conductor noise (A. van der Ziel); (7) Noise in transistors (W. H. Fonger).

The importance of noise in electron devices needs no emphasizing and this survey by a group of experts will be welcomed by many. This is primarily a book for the expert. It contains a valuable fusing of basic fundamental mathematical analysis of fluctuation phenomena, with an enormous amount of practical 'know-how' regarding the avoidance of noise. The sections are independent and weightily documented. The illustrations throughout are attractive but particularly well illustrated is the section dealing with low-noise travelling-wave tubes, a chapter which occupies the best part of a hundred pages.

The two last chapters (semi-conductors and transistors) although brief, should attract a very wide circle of readers.

Despite the formidable price, this book is an essential to all research workers engaged in electronics, especially to those working with computors, transistors, circuitry or high sensitivity systems.—S. TOLANSKY.

NEW DEVELOPMENTS IN THE PHYSICAL CHEMISTRY LABORATORY

by S. Lewin, M.Sc., Ph.D.

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PART VI—THE USE OF BORAX-MANNITOL SYSTEMS FOR BUFFERING PURPOSES

MANY physico-chemical investigations require constant pH conditions; others require also constant ionic strength[1] over the entire pH range of the investigation. In some cases it is desirable to cover an experimental pH range of as many as four to five pH units.

The first requirement is readily attainable. For this one needs a solution containing a weak acid plus its salt (A), or a weak base plus its salt (B), which has the requisite pH value. If (A) is used, e.g. acetic acid plus sodium acetate, then the Henderson-Hasselbalch equation, in the form of activities, may be employed to calculate the required quantities

$$pH = pK_a + \log \left\{ (a_{salt})/(a_{acid}) \right\} \quad \ldots\ldots\ldots(1)$$

which, when activity coefficients are justifiably neglected, may be written in terms of concentrations, thus

$$pH = pK_a + \log \left\{ [salt]/[acid] \right\} \quad \ldots\ldots\ldots(2)$$

When (B) is used, e.g. NH_4Cl plus NH_4OH, the corresponding equation, when activity coefficients approach unity, is

$$pH = (pK_w - pK_b) - \log \left\{ [salt]/[base] \right\} \quad \ldots(3)$$

pK_a, pK_b and pK_w represent respectively the pK values of acid, base and water. The required pH values of a given type of buffer may therefore be obtained by suitable variation of the above ratios of the particular acid-salt or base-salt pairs.

However, variation in the concentration ratio in a given (A) or (B) buffer results in ionic strength changes owing to the 'weak' partner, viz. weak acid in the (A) pair, or weak base in the (B) pair, being un-ionized, while the corresponding partner is completely ionized.* Such ionic strength changes are often undesirable because the properties of the buffered medium alter with ionic strength, at constant pH, e.g. rates of acid-base catalysed reactions. The accepted method of forming a series of solutions of varying pH values at constant ionic strength is to add a third component, viz. a strong electrolyte such as KCl, the concentration of which is decreased as the ionic concentration of the buffer salt is increased with rise of pH, in such a way as to keep the total ionic concentration constant.[2]

The buffering capacity of a given medium may be considered in terms of the expression $\frac{dA}{dpH}$ or $\frac{dB}{dpH}$, where dA and dB represent the quantities of acid or base involved, i.e. in terms of the quantity of acid or base required to cause a specified variation in the pH value of the medium.

Consideration of equations (2) and (3) shows that the buffering capacity of an (A) or (B) buffer is greatest at the midpoint of the neutralization curve[3], the effect decreasing away from the pK value and that the buffering capacity decreases to such an extent outside the pH range between ca. (pK + 1.1) and (pK — 1.1) that the use of the system for buffering purposes outside this range is, in most simple cases, questionable.[4] Indeed, it would be advisable to limit the experimental pH range to lesser values than the above range of 2·2 pH units as the buffering capacity requirements become more stringent. It is, of course, possible to obtain an extended pH range by using a suitable polybasic acid, or polyacidic base, and the corresponding salt system whose various pK values are such that a range of as many as four pH units may be covered, e.g. citric acid/citrate mixtures. However, considerable increase in ionic strength value results from rise in pH because the salt passes from a singly charged anionic state to a

[1]The molal ionic strength is by definition (Lewis and Randall, 1923) $\frac{1}{2}\sum m_i z_i^2$, while the molar one is $\frac{1}{2}\sum C_i z_i^2$, where m_i is the ionic molality, c_i is the ionic molarity, and z represents the magnitude of the charge. However, as pointed out by the author ('The Solubility Product Principle', Appendix on Ionic Strength, Pitman & Sons, In the press), appreciable differences in electrical field strengths may exist in solutions having the same 'Ionic Strength', when the above definitions of Lewis and Randall are followed.

*In other cases the 'acid' is charged, but the salt partner is charged to a greater extent, e.g. a phosphate buffer, containing $H_2PO_4^-$ and $HPO_4^=$

[2]Thus, suppose we wish to prepare a series of solutions of acetate buffers over a given pH range, at constant ionic strength, we may start at the lowest pH value with a given ratio of acetic/acetate plus a given concentration of an inert electrolyte, such as KCl. As the acetate concentration is increased along the series, the concentration of the indifferent electrolyte is reduced by an amount equivalent to the ionic strength increase associated with the increase in the acetate concentration.

[3]The experimental form of the curve may differ considerably from the theoretically deduced one when it passes below pH 4 or above pH 10. This is due to the water itself; a water correction curve must be applied in such regions.

[4]Consider, for example, the following values for the variation of pH, at 20°C., on addition of 0·2 N NaOH to 50 ml. of 0·2 M KH_2PO_4 and dilution to 200 ml.

pH	ml. 0·2 N NaOH	d(ml. 0·2 N NaOH)/dpH	
5·8	3·66		
		≈1·98/0·2	at pH 5·9
6·0	5·64		
6·8	23·6		
		≈6·0/0·2	at pH 6·9
7·0	29·6		
7·8	45·2		
		≈1·6/0·2	at pH 7·9
8·0	46·8		

Thus, the buffering capacity decreases by over three times in going from the pK region towards the opposite ends of the neutralization curve.

trivalent one; and the effect of the charge is squared so far as ionic strength is concerned.

A mixture covering more than one acid-base pair which covers a considerable pH range may be used, e.g. The 'Universal Buffer' of Michaelis (1931) containing acetate, veronal, NaCl and HCl. This buffer extends over the pH range of 2·6 to 9·6 and possesses constant 'ionic strength', (as defined by Lewis and Randall (1923)) and adequate buffering capacity. However, veronal possesses acid -NH groups[5] capable of reacting with formaldehyde (Lewin, 1956c) and presumably with other aldehyde groups[6] which reactions may, of course, lead to complications. Hence, the limitations of this buffer should be borne in mind. However, probably no other buffer mixture is free from criticism.

Reviewing the present categories of buffer solutions covering given pH ranges, it can be seen that pH variation is achieved by the addition of weak acids to their salts, or weak bases to their salts, or direct addition of acid or alkali to the corresponding partners. However, on the basis of available data and further experimental work carried out by the writer, it is advocated that complex formation between neutral molecules and constituents of *single* buffer systems be used as a basis for preparation of a series of buffer solutions to cover pH ranges wider than those covered by addition of acid or alkali to the single buffer system. Such systems can be arranged to be at constant 'ionic strength' without the addition of inert electrolytes.

Thus, suppose we start with an aminoacid buffer such as that of glycine in the pH region above that of its isoelectric point say at pH 8·5 and that we add to it increasing quantities of an aldehyde.[7] Then the pH values of the resulting solutions will be progressively lower than that of the initial solution of the aminoacid. This phenomenon is adequately covered in simple cases by the reactions

$$-NH_3^+ \rightleftharpoons -NH_2 + H^+$$

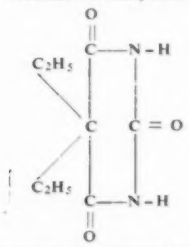
$$-NH_2 + RCHO \rightleftharpoons -N(H)-C(R)(H)-OH$$

$$-NH_2 + 2RCHO \rightleftharpoons -N(CH(R)OH)(CH(R)OH)$$

which result in increase in hydrogen ion concentration. (See also Lewin, 1956a, 1956c and Lewin and Kosinski, 1958). The resulting pH values are comparatively stable, over several weeks at 20°C. under anaerobic conditions. The pH depressions which may be obtained on the addition of increasing quantities of formaldehyde to a given aminoacid solution, at a fixed pH in the alkaline range, may reach as high a value as 3 pH units, giving therefore an equivalent pH buffer range with a constant 'ionic strength'. Such buffer systems, possessing free formaldehyde in equilibrium with the formaldehyde-aminoacid anion complex, are bacteriostatic; this is obviously advantageous in certain cases. However, the use of aminoacid-formaldehyde solutions for buffering purposes is disadvantageous in several respects, as will be seen presently.

In preparing any buffering system, the ease of preparation of the solutions and their degree of stability are of paramount importance. The ideal requirement is a buffer solution which will retain its stability over many months, or the period covering the overall investigation. If the final buffer solution is not itself stable over long periods, then the 'parent' solutions, from which it can conveniently be made by mixing, should be stable[8]. However, the stability of both the aminoacid solutions and that of formaldehyde is somewhat limited. Alkaline aminoacid solutions tend to break down progressively faster with pH rise[9], and formaldehyde solutions tend to oxidize or polymerize

[5]Veronal, viz. diethylbarbituric acid, may be written as:



However, the tautomeric equilibria involved in the various carbonyl/imino group interactions, viz.

$$-C(=O)-N(H)- \rightleftharpoons -C(-OH)=N-$$

result in *several* possible tautomeric forms.

[6]This problem is being investigated, and the results will be published in due course.

[7]Formaldehyde and other substances capable of possessing an aldehyde group, e.g. glucose, behave as non-electrolytes below ca. pH 8·5 at 15°C. and at lower temperatures. This approximate pH limit is lowered by ca. 0·2 to 0·3 pH units per 10°C. rise in temperature. Above these pH values, these substances behave as weak acids, the titration of which follows the Henderson-Hasselbalch Equation. Further, in the higher pH region transformations of the sugars, e.g. glucose into fructose, take place probably via their anions (See, for example, Lewin (1957)).

[8]Certain buffer tablets—which when dissolved in the stated quantity of water give rise to a definite pH—are commercially available. But, so far, only borax, phosphate at pK_2 value, and potassium hydrogen phthalate tablets are available.

[9]Under anaerobic conditions, e.g. storage under nitrogen free from oxygen, the aminoacid solutions, at slightly alkaline pH values, e.g. up to pH 9 tend to retain their pH values for nearly two months at 20°C. However, rise in temperature decreases their stability considerably (See Lewin (1956a) and Lewin and Kosinski (1958)). For this reason it is advocated that aminoacid buffer solutions, once prepared, should be kept in a refrigerator.

sometimes.[10] Further, the preparation of formaldehyde solutions of a given initial concentration is inconvenient, because it requires several volumetric determinations instead of simple weighings.

For these reasons the use of formaldehyde-aminoacid solutions cannot be considered convenient for universal use as buffer solutions having constant buffering capacity and constant 'ionic strength'.

Instead of using formaldehyde, reducing sugars may be employed, giving rise to aminoacid-aldose interactions; the sugars, being solid, may be readily weighed and made up in water to standard solutions. The interaction of aminoacids and reducing sugars also results in pH depressions; but the values of the depressions are considerably smaller than those caused by the formaldehyde interactions. Further, the attainment of the final pH depression value when formaldehyde is used is comparatively rapid—often a matter of minutes—the reaction with hexoses is comparatively slow, taking at times as much as two days at 20°C. Pentoses may be employed instead, when the reaction—while slow compared with formaldehyde interaction—is much faster, a steady pH state being reached within an hour at 20°C. However, whether hexoses or pentoses be used and a steady pH state is reached, a new phenomenon is noted. The steady pH state which has been attained at first remains steady and reproducible for some time (see Lewin (1956a, 1956b), Lewin and Fox (1956), Lewin and Kosinski (1958)). However, there follows a series of subsequent steady pH states, each with progressively greater pH depression value, all of which are reproducible; and though the series of data have been recorded, it is clear that such systems—important though they are so far as the aminoacid-sugar interactions are concerned—cannot reasonably be considered convenient so far as constant buffering capacity and constant 'ionic strength' buffer requirements are concerned.

The next category of possible buffer combinations to be examined was that of borate-polyhydroxy compound mixtures. The reaction between borate and polyhydroxy compounds such as glycerol, glucose or mannitol results in considerable pH depressions which may amount to over four pH units (see Hildebrand, 1913). For example, on mixing, at 20°C., 0·05 M sodium borate ($Na_2B_4O_7.10H_2O$), pH 9·18, and mannitol solutions in equal proportions giving rise to a final overall mannitol concentration of over 0·4 M, the resulting pH values are below pH 5. For the purpose of standard solution preparation, the weighing of solids such as glucose or mannitol is preferable to the use of liquids such as glycerol, and hence the glucose-borate and mannitol-borate interactions were examined. The borate-mannitol and borate-glucose interactions have been investigated previously by several workers from the standpoint of molecularity (see Böeseken, Vermaas and Küchlin (1930); Van Liempt (1920)) and from the standpoint of estimation of boric acid by conversion into a stronger acid capable of titration to an endpoint with phenolphthalein (e.g. Dodd (1929, 1930)). The investigation reported here was concerned with the buffering uses mentioned (details of related investigations will be published elsewhere).

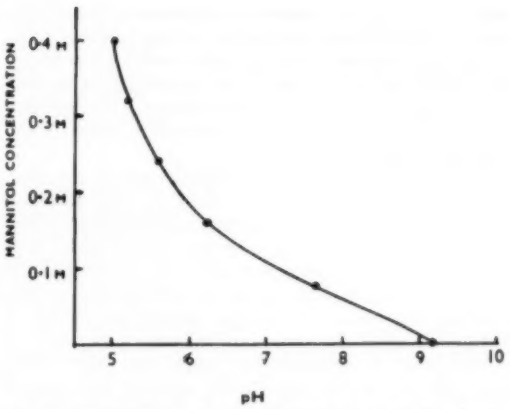


Fig. 1. Variation of the pH depression values in borate-mannitol solution with mannitol concentration at 20°C.

The series of borate-polyhydroxy compound mixtures prepared contained a fixed quantity of borate (0·025M) and varying proportions of the polyhydroxy compound. They were prepared by mixing fixed volumes of a borax solution (0·05M $Na_2B_4O_7.10H_2O$, Analar) with x ml. of the polyhydroxy compound (0·8 M in the case of mannitol, 1·5 M in the case of glucose) and y ml. of CO_2 free distilled water, $(x+y)$ remaining constant) The solutions were placed in glass stoppered tubes and allowed to attain the temperatures of the thermostats in which they were placed, viz. 20°C. and 40°C.[11] Small quantities were withdrawn at intervals and were subjected to glass electrode pH measurements which were carried out using thermojacket pH vessels (Lewin, 1953a) and Internal Bridge calomel electrodes (Lewin, 1953b). The measurements were sometimes carried out for over two months. Any glass electrode standardizations and calibrations necessary were carried out as described previously (Lewin, 1955). The pH values in the case of the borate-mannitol solutions, once temperature equilibrium was attained, remained steady over the experimental duration in all the cases examined, and in those solutions which were kept over three months at 20°C. No brown colour development was noted in the borate-mannitol solutions even after a month at 40°C. The pH values are plotted in Fig. 1.

The glucose-borate solutions gave rise to smaller pH depressions. This need not be a serious disadvantage so far as buffering requirements are concerned. However, whereas mannitol is comparatively inert, glucose reacts

[10]It is possible to stabilize formaldehyde solutions against oxidation by storage under nitrogen free from oxygen. However, the solutions tend to give rise to a precipitate of formaldehyde polymer, whether aerobic or anaerobic conditions be employed.

[11]About 0·1 ml. of toluene was added to each tube for bacteriostatic purposes.

with a number of substances. Further, slight yellow colour development—indicative of the onset of the 'browning reaction'—was visually noted after about two weeks at 40°C. when some instability in the pH values was recorded. For these reasons, the mannitol borate buffer system can be considered superior to the glucose-borate system.[12]

Provided we use the same 'parent' borate solution, i.e. having the same initial pH, for preparing the series of borate-polyhydroxy complexes, the buffering capacity should remain constant over the entire pH range involved. It is, of course, possible to use a different 'parent' borate solution, e.g. boric acid adjusted to, say, pH 9·6. The series of solutions obtained with such a 'parent' borate solution and varying quantities of polyhydroxy compound will have the same 'ionic strength' and the same buffering capacity throughout. However, this series will have a different buffering capacity and a different 'ionic strength' from the previous series. Now, the pH depression values are dependent on the mannitol concentration and practically independent of the original borate concentration. It follows that using 'parent' borate/boric mixtures with initial pH values from pH 8 to pH 10, the overall pH range between ca. pH 4 to pH 10 may be covered by interaction with mannitol. In such an arrangement the increase in range of pH covered is associated with loss of constant buffering capacity and constant 'ionic strength'. It is possible to obtain a constant 'ionic strength' borate/boric-mannitol-inert electrolyte buffer by suitable variation in the concentration of the inert electrolyte along the pH range as the pH of the initial parent boric/borate 'parent' solution is altered. Such an arrangement, though not possessing equal buffering capacity throughout the series, has a constant 'ionic strength' pH range of over 6 pH units and is likely to be of use in certain investigations.

The borate-polyhydroxy complex buffer systems have one particular disadvantage which may be considered serious under certain conditions, viz. that of dilution affecting the pH value, as the concentration of the mannitol is affected. Provided however that this limitation is observed, there are a number of uses for such solutions, particularly because of their ease of preparation and their stability. For example, they may be used for standardizing glass electrodes over the available pH range. It is essential to standardize glass electrodes at least at two points, preferably those covering the experimental pH range (Lewin, 1955). Usually potassium hydrogen phthalate and borate are used; occasionally phosphate is employed. As they cover the entire range of pH 5 to pH 9 we would suggest that the borate-mannitol mixtures should be used for glass electrode standardization, checking at four or five points along the range. The respective final buffer mixtures can be stored at room temperature for at least three months. Alternatively, the 'parent' borate solution and mannitol solution, which can be stored for at least one year at room temperature, can be mixed readily in accurate proportions, using pipettes or burettes as preferred.

Let us consider now the use of borate-mannitol complex formation from the standpoint of co-relating theory and practice during the teaching of practical physical chemistry. Complex formation is followed in the laboratory employing usually either the iodide-iodine system, viz. $I^- + I_2 \rightleftharpoons I_3^-$ or the cuprammonium system, viz. $Cu^{++} + 4NH_3 \rightleftharpoons Cu.4NH_3^{++}$. These systems require the preliminary determination, or assumption of, the respective partition coefficient values of iodine between water and chloroform and that of ammonia between water and chloroform. The experiments are tackled conveniently by the use of straightforward volumetric analysis involving the respective thiosulphate-iodine and ammonia-HCl determinations. It is probably the simplicity of the determinations which has led to these experiments having been widely quoted in physical chemistry books and having become standard experiments in physical chemistry teaching laboratories.

The above equilibria and the borate-mannitol system possess an outstanding feature in common, viz. interaction between charged radicles and neutral molecules, as it has been established that the borate ion reacts with two molecules of mannitol, thus $B^- + 2M \rightleftharpoons BM_2^-$ (Böeseken, Vermas and Küchlin (1930)).

It is pertinent to pose the problem whether given the very limited time available for the practical physical chemistry course, which may allow for only one such type of experiment, which experiment should be favoured. It must be emphasized that the practical physical chemistry course should not be planned merely from the standpoint of teaching practical details, it must be used to co-relate as many theoretical principles as possible with the practical details and limitations of their executions or application. The iodine-iodide and cuprammonium complex formations are of paramount importance, but their ramifications in the teaching of theory and practice of physical chemistry are not as wide as those encountered in the practical teaching of the borate-mannitol complexes. On the practical side there is the standpoint that iodine and cuprammonium complexes, determinations require volumetric measurements with which the students are familiar, as they are used repeatedly in the teaching of both inorganic, organic and physical analysis. The same applies to the simple preparation of standard solutions of the substances, which is common practice in the laboratory. On the other hand, the borate-mannitol interaction, if adopted as a standard physical chemistry laboratory experiment, offers considerably wider scope in both the theoretical and practical fields. On the theoretical side there is the opportunity of expounding both the principle of equilibrium attainment and the resulting effect of pH depression, thus

$HB \rightleftharpoons B^- + H^+$ $\qquad$ $B^- + 2M \rightleftharpoons BM_2^-$

and the associated concept of pH in its relation to the hydrogen ion concentration and hydrogen ion activity in the two respective definitions $pH = -\log[H^+]$, and

[12]Details concerning the investigation of other hexose-borate and pentose-borate interactions will be published elsewhere.

$pH = -\log(a_{H^+})$ including the errors involved in switching over from one pH scale to the other. The theoretical significance of glass electrode pH measurements and its relation to other types of pH measurements can be discussed. The effect of ionic strength on activity coefficients and therefore on pH values can be considered along with the whole field of buffering requirements as well as the titration of weak acids. On the practical side there is the technique of preparing a constant volume series, problems involved in the standardization and checking of glass electrodes, stability of solutions, and the use of toluene as a bacteriostatic agent.

Such an approach teaches the student the significant difference between an *experiment* which he rushes through merely to get the data required, and the concept of *investigation*, in which the pros and cons of both theory and practice are considered. In the long run, short as the time allotted for the practical physical chemistry course may be, the investigational approach is superior both from the overall educational aspect and the narrower standpoint of success in examinations. For these reasons, it is advocated that the borate-mannitol reaction should be included in the practical physical chemistry curriculum.

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APPARATUS AND DEVICES

A VERSATILE KARL FISCHER TITRIMETER

by F. B. Waddington

Research Department, Metropolitan-Vickers Electrical Co. Ltd.

Summary

Moisture is a common cause of electrical insulation failure. There is therefore a constant demand within the electrical industry for the accurate and reliable determination of moisture in a wide variety of materials.

A Karl Fischer titrimeter for chemically determining the moisture present in materials in amounts as low as one part per million is described. This apparatus incorporates several original features and has proved highly satisfactory in use during the past five years.

1. Introduction

The Karl Fischer reagent—a solution of sulphur dioxide, pyridine and iodine in methanol—is now well established as a powerful desiccant, which has a highly selective, quantitative and rapid reaction with moisture even in certain molecularly combined forms. The reaction may be presented in the following simplified form:

$$CH_3OH + SO_2 + I_2 + H_2O + 3C_5H_5N \rightarrow 2C_5H_5NHI + C_5H_5NHSO_4CH_3$$

This reagent (commercially available from B.D.H. Ltd.) (Stuckey, 1953) is fairly stable and has a shelf life of the order of several months. During the past few years there have been several suggested modifications to this reagent; these include a sulphur dioxide, bromine, chloroform mixture (Belcher and West,1953) claimed to be more stable and more stoichiometric in its reaction with water than the conventional reagent, and a two-solution Karl Fischer reagent (Seaman *et al*, 1949) which may be used as a standard iodometric reagent and which can be stored indefinitely. However, none of these is likely to prove as useful or as versatile as the original reagent.

To measure the water content of a material, the moisture must first be transferred into a suitable solution which is subsequently titrated with Karl Fischer reagent. This is readily achieved with a large number of materials simply by dissolving in methanol or other suitable solvent; with others the moisture can be removed by an alcohol washing technique or, as a last resort, by digesting with benzene or pyridine and distilling off the water azeotrope.

Some time ago, a need was felt for an apparatus which could be used for the quick and accurate determination of moisture by this titration method in a wide variety of materials; these included some only available in small quantities and others insoluble or highly coloured. A Karl Fischer titrimeter was therefore required which would comply with the following conditions of design and versatility:

1. The apparatus should be totally enclosed to avoid contact with atmospheric moisture, but should be readily cleaned without dismantling.
2. The titration vessel should be of such a design that large or small volumes could be titrated and insoluble materials introduced.
3. A sensitive device for detecting the titration endpoint was required so that moisture in coloured materials could be determined and traces of moisture measured with accuracy by comparatively unskilled personnel.

An apparatus to meet all these requirements was not

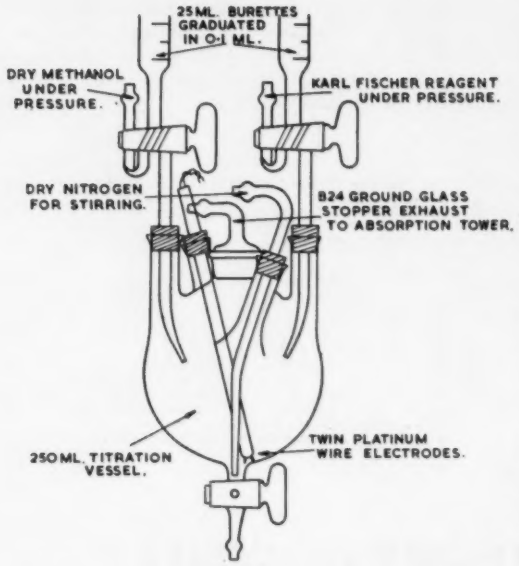
25 ML. BURETTES GRADUATED IN 0·1 ML.
DRY METHANOL UNDER PRESSURE.
KARL FISCHER REAGENT UNDER PRESSURE.
DRY NITROGEN FOR STIRRING.
B24 GROUND GLASS STOPPER EXHAUST TO ABSORPTION TOWER.
250 ML. TITRATION VESSEL.
TWIN PLATINUM WIRE ELECTRODES.

FIG. 1.
TITRATION FLASK ARRANGED FOR ROUTINE MACRO ESTIMATIONS.

commercially available. The following notes describe an apparatus—specifically designed to meet these requirements—which includes several novel features and which has proved most satisfactory during five years of laboratory use.

2. The Karl Fischer Titrimeter

A titration vessel was designed (Fig. 1) which, though having a fairly large volume of 250 ml. was—by virtue of its shape—suitable for titrating a volume as small as 0·5 ml. Ground glass joints were found to be very prone to breakage or seizure and also liable to entrap moisture (Roberts and Levine, 1949); these were therefore reduced to a minimum and replaced where possible by rubber stoppers fitted into extension necks (Fig. 1) to prevent contact with any solvents in the titration vessel. The rubber consequently had an almost indefinite life. The extension necks were so arranged that a dry nitrogen bubbler (Fig. 1), a twin platinum electrode, or a propeller-type stirrer (Fig. 4) could be inserted so as to reach the bottom centre of the titration vessel; this enabled small volumes of liquid to be titrated. The burettes were readily interchangeable; either 25 ml. burettes calibrated in 0·1 ml. (Fig. 1) or 2 ml. microburettes calibrated in 0·01 ml. (Fig. 4) were used. To enable the titration vessel to be cleaned without dismantling, a stopcock was attached as close as possible to the base of the vessel.

Samples were introduced in one of three ways: (*a*) by pouring or pipetting directly into the titration solvent; a B. 24 ground glass stopper was placed at the centre of the titration vessel for this purpose (Fig. 1), (*b*) by using a graduated reservoir (Fig. 4); this was essential for accurate work to ensure that no moist air was admitted with the sample, (*c*) insoluble materials were placed in a washing vessel containing a ground glass filter (Fig. 2) and the pretitrated solvent (i.e. moisture free) used for washing was passed in through the base stopcock of the titration vessel using dry nitrogen under pressure.

One reagent burette was employed for the Karl Fischer reagent, the other for dry methanol—this was convenient for use as a solvent in all titrations—and both burettes were filled by means of pressure obtained from a nitrogen cylinder. Conventional methods for drying the nitrogen and for preventing ingress of moisture into the apparatus were employed, i.e. drying tubes for the burettes and drying towers for the nitrogen supply—both packed with anhydrous magnesium perchlorate.

The electrometric end-point detection circuit (Fig. 3) was of the simple form for 'dead stop' direct titration first suggested by Cornish (1946), but with the following innovations: the two platinum electrodes were incorporated into one insert; the applied potential across the platinum electrodes could be varied over the range 0-130 mV. (essential for the titration of a wide range of solvent mixtures); and a sensitive though robust microammeter was used which could be read to 0·5 microamp.

3. Manipulations

Several hundreds of compounds and complex materials have been successfully directly titrated for moisture by many independent investigators; these compounds include many inorganic materials and an extremely large number of organic compounds ranging from simple paraffins to complex proteins and antibiotics. An excellent detailed account of this wide range of directly titratable compounds is given in a monograph by Mitchell and Smith (1948). Many further compounds which interfere (i.e. react with the Karl Fischer reagent) can still be directly titrated using modified techniques, for example, water in the presence of ferric salts can be directly titrated if the iron is first complexed with 8-hydroxy quinoline (Laurene 1952). Compounds which interfere, and for which modified techniques are not available, appear to be the exception rather than the rule, for example, although no method is available for the titration of diacyl peroxide, dialkyl peroxide and hydroperoxide can be directly titrated (Mitchell and Smith 1948).

Difficulties arise in this direct titration method when the material is in the form of a liquid which is immiscible with Karl Fischer solvents or is an insoluble solid. The technique of digesting the material with benzene or pyridine and distilling off the water azeotrope, which is subsequently titrated, has been successfully used by various investigators (Roberts and Levine, 1949, Mitchell, 1951, Meelheim and Roark, 1952); but this is a difficult time-consuming manipulation and is entirely unsuitable for thermally unstable materials. The following is a brief description of two simple methods which have been successfully used to overcome this problem.

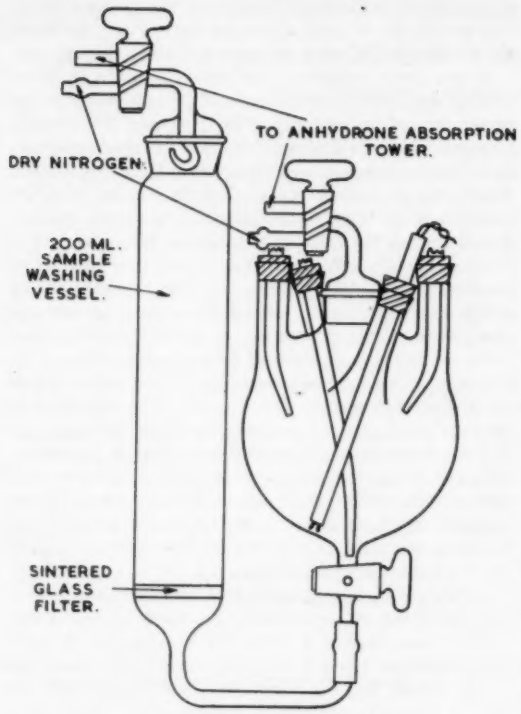

FIG. 2.
ARRANGEMENT FOR EXTERNAL EXTRACTION.

For immiscible liquids a solvent mixture was employed which was suitable for the Karl Fischer reagent and its reaction products, and for the electrometric detection of the end-point, yet also enabled the immiscible liquid to be emulsified by a gaseous (Fig. 1) or mechanical (Fig. 4) mixing device. Of particular interest, using this method, was the estimation of moisture in a wide range of insulating oils. In agreement with Acker and Frediani (1945) it was found that a mixture of 20 parts methanol with 50 parts of chloroform was a good solvent for Karl Fischer reagent and also enabled up to 50 parts of oil to be emulsified by moderate gaseous or mechanical agitation. Furthermore it was noted that efficient emulsification of the oil was most essential if the Karl Fischer reagent was to have effective access to the moisture in the oil. It was also found that the electrometric direct titration end-point was most sensitive for the above solvent mixture with a potential of 90-100 mV. across the electrodes whence, in the region of the end-point, an equivalence of 1 microamp per 0·01 ml. Karl Fischer reagent (i.e. approximately 0·04 mgm. water) could be readily obtained. However, the accuracy of this determination was limited by the stability of the stirring device employed, and gaseous stirring was certainly not sufficiently stable to utilize this full sensitivity; the erratic depolarizing effect of the gaseous bubbling produced a fluctuation of the order of 5 microamps which was equivalent to an error of $\pm$ 2 parts per million on a 50 ml. sample. The specially designed mechanical double stirrer (Fig. 4) enabled violent agitation (and therefore efficient emulsification) of a sample in the upper layers whilst the small terminal propeller ensured a smooth circulation of the liquid over the electrodes. Using this stirrer the galvanometer reading was stable to 0·5 microamp and the full sensitivity of the electrometric end-point could therefore be utilized; this gave an accuracy of $\pm$ 0·2 p.p.m. on a 50 ml. sample of oil. Using this latter technique it was found that momentarily opening the titration vessel to introduce a sample resulted in large errors, for example, the ingress of moist air during the introduction of a 50 ml. sample of oil resulted in the addition of the order of 1 p.p.m. to the moisture value of the oil. The closed system of introducing the sample (Fig. 4) was therefore employed.

The moisture content of solid insoluble materials such as textiles, mica, papers, fillers and the like was estimated by means of an extraction attachment (Fig. 2) which enabled the surface and imbibed moisture to be removed by washing with a pretitrated solvent. A slight pressure of dry nitrogen was used to transfer the titrated solvent from the titration flask into the washing vessel and back, after which the titration was repeated until the system was moisture-free; the sample was then introduced into the washing vessel, the solvent returned, and the passage of dry nitrogen through the sintered glass disc produced an efficient washing action in the vessel.

In all the above manipulations the titration end-point was detected as follows: With a suitable quantity of solvent or solvent mixture in the titration vessel, a constant rate of mixing, and a suitable potential across the platinum electrodes, the Karl Fischer reagent was added until a permanent deflection of 50-80 microamps was observed on the microammeter. The precise deflection was noted. The sample was then introduced (or the solvent transferred to the washing vessel and returned) and then further Karl Fischer reagent added

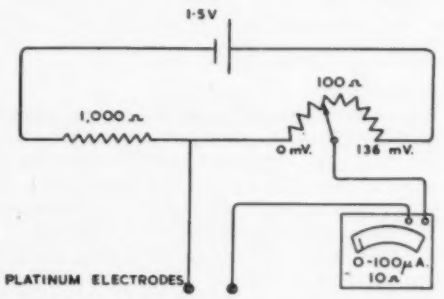

FIG. 3.
CIRCUIT FOR THE ELECTROMETRIC DETECTION OF THE END POINT.

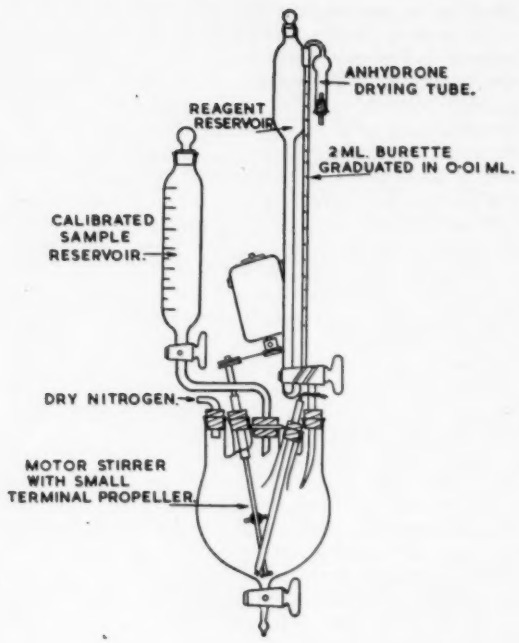

FIG. 4.
ARRANGEMENT OF THE APPARATUS FOR MICRO ESTIMATIONS.

until the microammeter returned to its original reading. If an excess of reagent was accidentally added, the accurate titre could be readily interpolated from the microammeter reading. A rapid standardization of the reagent was then made by adding 0·01 ml. of water from a micro-pipette to the moisture-free system containing the sample and solvents and once again adding the reagent to return the microammeter to the original deflection. If, perchance, the resistance of the titration cell was so increased by the addition of the sample that the original deflection could not be reached, the titration was repeated using a lower (10-20 microamps) setting of the galvanometer.

4. Applications

Throughout the five years that the apparatus has been in use, there has been a continual demand for moisture determinations of a character placing them well outside the scope of former techniques (e.g. oven drying, Dean and Stark distillation). These include the determination of traces of moisture in the following materials: a wide range of industrial and pure solvents, for example ethyl and propyl alcohols, benzene, white spirit and glycerol; a variety of petroleum products including insulating oils, greases, and lubricating oils; insulating materials, such as bakelite bonded paper board and insulating varnishes; also fine powders, litharge for example (for use in glycerine/litharge cement) and coal dust which were examined for surface moisture. An interesting application was the use of the Karl Fischer titrimeter to evaluate the efficiency of a plant for drying transformer oil.

It has been observed that there is often a direct relationship between moisture content and the electrical properties of a material. For example, the electric strength of an insulating oil usually varies inversely as its moisture content, and the power factor of a silica filled silicone rubber varies directly as the moisture content of its filler. However, such a simple relation is not always the case, and an explanation for this is that, whereas small quantities of moisture present as droplets or surface layers—and so constituting an additional phase—will undoubtedly worsen the dielectric properties, water present in a combined form (e.g. water of hydration) may not do so. Furthermore, even the shape of water droplets can have an important effect on dielectric properties (Sillars 1937). The Karl Fischer reagent does not normally differentiate between the various forms in which moisture is present in a material, and this is on the whole an important advantage in that, under certain conditions of elevated temperatures or high electric stress, combined moisture or inactively imbibed moisture may revert to free moisture and so promote an insulation failure.

As shown by a large number of investigators the Karl Fischer form of aquameter can have many further applications; moisture in gases (Waddington 1957), in paints (Swann 1946), and in petroleum fuels (Loveland *et al.*, 1958) can be accurately determined, and the course of many chemical reactions where moisture is evolved or absorbed may be followed.

ACKNOWLEDGMENTS: *The author wishes to thank Sir Willis Jackson, F.R.S., Director of Research and Education, Metropolitan-Vickers Electrical Co. Ltd., for permission to publish.*

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APPARATUS AND DEVICES

It is evident from letters received that the Apparatus and Devices Section of *Laboratory Practice* arouses considerable interest. We should like to point out that we are at all times pleased to receive from readers notes, however short or simple, for inclusion in this section.

A Simple Urino-Faecal Separator for use in Experiments on Diuresis

by R. T. Brittain, B.Pharm., M.I.Biol.

Research Division, Allen and Hanburys Ltd., Ware, Herts.

THE usual procedure in experiments on diuresis is to compare the volume of urinary excretion of groups of normal and treated animals. In prolonged experiments use is made of metabolism cages with openwork floors under which are funnels with varying devices for separating urine from faeces (Lipschitz, Hadidian and Kerpscar 1943, Hansard and Comar 1953, Draper and Robins 1956). In the course of an investigation into the diuretic activity of some drugs in rats, a simple urino-faecal separator has been developed. (Fig. 1).

The apparatus consists of a circular metabolism cage of diameter 22 cm. of 0·8 cm. galvanized wire mesh, supported by three perspex ledges in the top of a large plain glass funnel. The oblique portion of the funnel stem has been extended to one side, leaving an aperture through which the faeces can fall into one arm of a dual glass receiver. The urine flows along the extended portion of the funnel stem and is collected into the other arm of the receiver. Both arms of the receiver are 2 cm. in diameter; of the two, the faecal arm is slightly higher and has been lipped to assist faecal collection. The incorporation of feeding and drinking troughs as attachments to the cage can be made as required.

Using groups of four to five rats (120-200 g.) the apparatus has been found to collect urine effectively and rapidly with no visual faecal contamination. The design of the apparatus is such that the surface area exposed to excretions is small, evaporation losses are low, and cross contamination of urine and faeces is minimized. The apparatus is simple to use, and can easily be reproduced for experiments involving large numbers of animals.

I would like to thank the Directors of Allen and Hanburys Limited and Dr. P. F. D'Arcy, Head of the Pharmacology Department, for permission to publish this communication.

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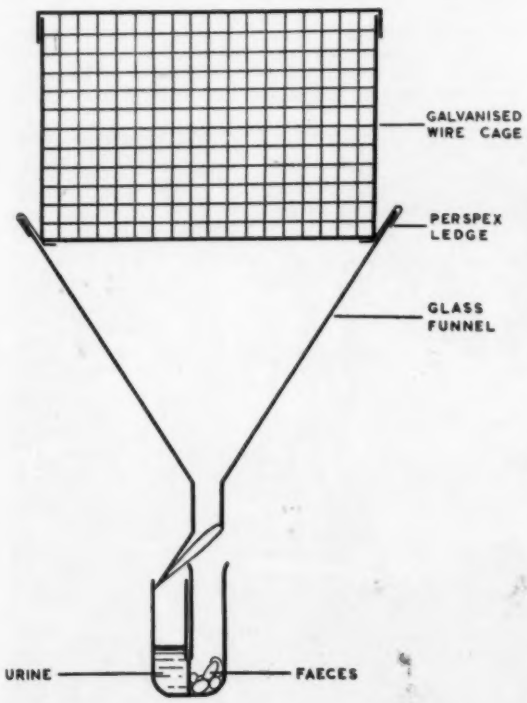


Fig. 1.

Paul Instrument Fund Grants

Awards of grants by the Paul Instrument Fund Committee have been made as follows:

£1,000 to Dr. H. B. Barlow, assistant director of research, department of physiology, and Mr. P. E. K. Donaldson, technical officer, physiological laboratory, Cambridge, for the development (*a*) of a device for automatically improving coding of messages and (*b*) of a diffused storage sequence engine, the object being to advance knowledge of the operation of comparatively simple assemblies of nerve cells by making instruments which perform the same task as such assemblies.

£600, in supplement of a previous grant, to Dr. E. T. Hall, senior research officer at the Research Laboratory for Archaeology and the History of Art, Oxford, for improvements to an apparatus with which magnetic measurements may be made with a view to dating archaeological material.

£2,000 to Dr. H. Motz, reader in engineering science, University of Oxford (in association with Prof. G. B. Walker, professor of electrical engineering, Essex College, Assumption University, Windsor, Ontario) for the construction of a linear accelerator working at 1·6 cm. (J-band).

£5,500 to Prof. R. O. Redman, professor of astrophysics in the University of Cambridge, for the construction and testing of a thin astronomical mirror of plate glass and of a new type of support system.

£5,900 to Dr. P. M. B. Walker, Royal Society Research Fellow, department of zoology, the Ashworth Laboratory, University of Edinburgh, for the construction of a new microspectrophotometer so that it will integrate over a defined but irregular area which can be altered quickly and easily.

DAIRY SCIENCE AT THE UNIVERSITY OF ALBERTA

THE title of the Department of Dairying in the University of Alberta has recently been changed to the Department of Dairy Science. In the past many graduate students have obtained a Masters degree as a result of research work in the department, but up till the present no graduate students in the department had taken the degree of Doctor of Philosophy.

Mr. John M. de Man, who received his training in analytical chemistry, chemical engineering and dairy science in Holland, has been doing postgraduate work in the department for the past four years and has successfully completed all the requirements for a Ph.D. degree and will shortly be recommended for this academic qualification. Mr. de Man's work has been a fundamental study of the physical properties of butter and the practical application of this knowledge to the 'spreadability' of butter, with particular attention to continuously made butter. For this work Mr. de Man has received two special awards for meritorious research from the Alberta Dairymen's Association.

Much of this work has been done jointly with Prof. Wood under the general direction of Prof. Thornton, the retiring head of the department. It is the intention that this work shall continue.

Mr. de Man has taken up duties as Assistant Professor in the Department of Dairy Science and with this appointment there will be a slight re-organization of the teaching in the department. The courses in Dairy Science will be divided into four sections as follows:

1. DAIRY TECHNOLOGY (Asst. Prof. F. W. Wood)

Dairy Technology.—The scientific and technical principles involved in the commercial manufacture of dairy and some other food products.

Principles of Hygienic Milk Production.—Farm control of quality with special reference to bacterial and flavour defects. Detergency and utensil sterilization. Pasteurization of milk in the farm home (a course specifically designed for General Agriculture and Animal Husbandry students).

Review and Literature Study.—(for senior students).

Advanced Dairy Science.—(research programmes for graduate students).

2. DAIRY AND FOOD BACTERIOLOGY (Prof. L. F. L. Clegg)

Agricultural and Food Bacteriology.—The relationships, characteristics, and metabolism of micro-organisms concerned with agriculture and allied industries, such as the fermentation industries, food processing (canning, freezing radiation) industrial effluents, etc. and the scientific principles involved.

Dairy Bacteriology.—The bacteriology of the production and distribution of fluid milk and cream and consideration of some of the problems of the market milk industry. Milk supplies and public health. The bacteriology and biochemical aspects of pasteurization. The bacteriology of butter, cheese, ice cream and other milk products.

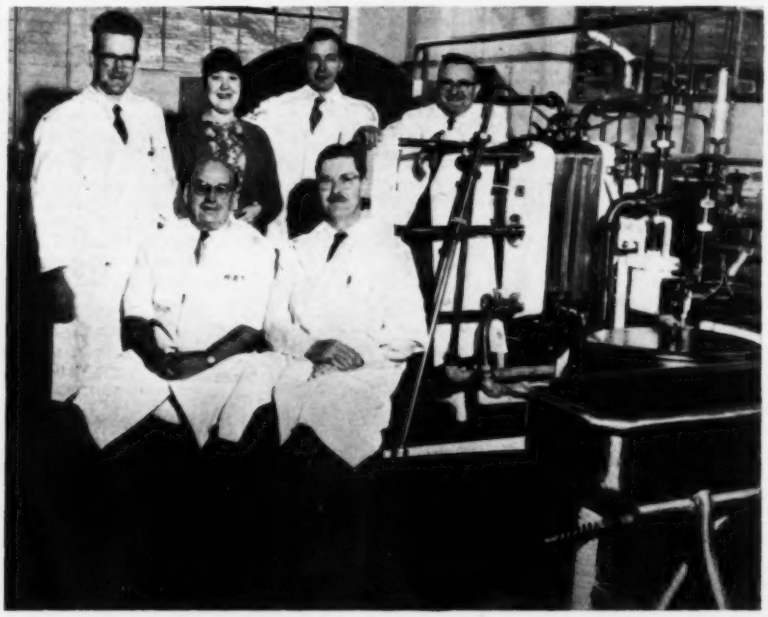
Review and Literature Study.—(for senior students).

Advanced Applied Bacteriology.—(research programmes for graduate students).

3. DAIRY AND FOOD CHEMISTRY (Asst. Prof. J. M. de Man)

Dairy Chemistry.—The chemistry of the major and minor constituents of milk and its products. The physiology and biochemistry of milk formation and secretion. Emulsions and foams. Enzymes and enzyme reactions in milk products.

Advanced Food Chemistry.—(for senior and graduate students). The relation of food constituents to industrial processes. Enzymic and non-enzymic deterioration reaction



Staff of the Dairy Science Department. *Standing, L. to R.:* **Asst. Prof. J. M. de Man, Miss Irene Short (Secretary), Mr. S. Rowe (Technician), Asst. Prof. F. W. Wood.** *Seated:* **Prof. H. R. Thornton, Prof. L. F. L. Clegg (Head).**

in foods. Surface chemistry. Flavour and sensory testing. Rheology of foodstuffs.

Review and Literature Study.—(for senior students).

Advanced Dairy and Food Chemistry.—(research programmes for graduate students).

4. DAIRY SCIENCE (Prof. L. F. L. Clegg and staff)

Introduction to Dairy Science.—An introduction to the economics, chemistry, physics, bacteriology, physiology, biochemistry and legal aspects of milk production.

Seminar.—Reports and discussions of research problems by graduate students.

Research and Scholarships

The laboratories of the department are well equipped for research in any of the above four sections. Research in the department receives financial support from such bodies as The Department of Agriculture, Ottawa; The National Research Council of Canada; and the National Defence Research Board. Such grants-in-aid provide for a limited number of assistantships. The assistants if eligible are permitted to register in the Faculty of Graduate Studies and the research findings may be presented to the University as M.Sc. or Ph.D. theses. Various Graduate Fellowships and Scholarships such as those outlined by the above-mentioned organizations are tenable in the department. During the last ten years some 30 scientific papers by members of the department have been published.

The Alberta Dairymen's Association Scholarship in Dairying of the value of $500 is open to undergraduates who have completed the second year in the Faculty of Agriculture and who are registered in a special programme in the Department of Dairy Science. The award is for scholarship attainment during the first two university years with a two-year average of not less than 75 per cent. The Scholarship may be renewed during the fourth year for continued meritorious work.

The Alberta Dairymen's Association also is considering support for students who intend to become dairy specialists and who have completed one summer's practical work in the dairy industry.

New Creep Information Centre

In response to requests from manufacturers and users of high-temperature materials, a Creep Information Centre is being set up at the National Engineering Laboratory (formerly the Mechanical Engineering Research Laboratory) at East Kilbride, so that industrial designers can more readily obtain data on the strength of high-temperature materials. It is hoped that it will become the national centre for information on the strength of high-temperature materials. All interested organizations are invited to co-operate in supplying experimental data so that the scheme may become as effective as possible.

The main object of the Centre will be to collect and tabulate all the available data on creep and rupture properties of conventional high-temperature materials. Data for British materials will be issued in an agreed form as far as considerations of commercial security allow. The scheme will later be extended to include fatigue and other mechanical properties of high-temperature materials and information about test procedures and testing machines; data on foreign materials may also be included.

A leaflet giving further details of the scope of the Creep Information Centre is available from the Laboratory. All firms and research organizations concerned with the production or use of high-temperature engineering materials are invited to participate, and to write to the Director, National Engineering Laboratory, East Kilbride, Glasgow.

The Institute of Physics

At the Annual General Meeting of The Institute of Physics held in London on July 7, 1959, Sir George Thomson (Master of Corpus Christi College, Cambridge) was re-elected President.

Dr. J. M. A. Lenihan was elected a Vice-President; Dr. J. Taylor, Honorary Treasurer, and Prof. F. A. Vick, Honorary Secretary, were re-elected. The two new Ordinary Members of the Board elected were Dr. V. E. Cosslett and Mr. L. Rotherham.

The Report for 1958 which was adopted at the meeting shows that at the end of the year the total membership in all grades was 6,309, an increase of 415 on the previous year. Eight hundred and fifty-one applications for election or transfer to the various grades were considered, which was 7 per cent more than the previous year.

The Report records that at the end of the year a circular was sent to members proposing the amalgamation of the Institute and The Physical Society and that if the broad basis of intention was accepted a Joint Committee would be set up to work out all the details; it is understood that a Scheme for amalgamation is about to be submitted to members of both bodies.

Eighty candidates took the recently established Graduateship Examination, and 26 satisfied the examiners. In 1958, 637 students took the Ordinary National Certificate examination and 246 the Higher National Certificate examination in applied physics (compared with 542 and 182 respectively in 1957).

Besides the normal work of the Institute the Report records expanding activities in many fields including education, scientific publications, conferences and symposia and other meetings arranged by the Institute's Branches at home and overseas and by its specialist subject Groups.

Thawing of Frozen Fish

The industrial thawing of frozen fish, which normally takes up to 24 hours in air can now be done in about 15 minutes by dielectric heating. This new development, which solves a major problem of the fish industry, is the result of work at the Torry Research Station of D.S.I.R. It is of great economic importance, especially in view of the steadily increasing large-scale utilization of frozen fish, and may have wide application in other sections of the food industry.

The method, known as dielectric thawing, depends on the fact that if any material is placed between, but without touching, two metal plates which are charged with an alternating voltage of many thousands of volts at a frequency of about 40 million cycles per second, energy is produced in the material in the form of heat. Under well-defined conditions, fish may be uniformly thawed throughout a block of the frozen material—either as whole fish, as in the case of herrings, or as fillets. It is possible to control conditions far more accurately than in existing methods, for example, so that individual fish in a block may be separated while remaining partially frozen.

Laboratory-scale experiments at the Torry Research Station, Aberdeen, had shown that it was possible to use dielectric heating successfully on tiny pieces of frozen fish. Pilot scale apparatus, using slightly modified equipment which is commercially available, was therefore set up. Initially, the problem of 'runaway heating' was encountered; small portions of blocks of fish absorbed the major part of the available energy and became cooked, while the rest of the block remained hard frozen.

This problem has now been solved, and fish may be fed into the machine on an endless belt and thawed in 15 minutes.

NEW FOOD FACTORY LABORATORIES

In building a new food factory at Kitt Green, Lancashire, H. J. Heinz Company Ltd. have had an opportunity to put into practice experience gained during many years of food production not only in England, but also in the United States, Canada and other countries of the Commonwealth. This experience has shown itself as much in the layout and design of the laboratories as it has in the design of the Production Departments.

IN the laboratories first consideration has been given to the amount of space allotted to each function. Since food production is greatly dependent on the control of micro-organisms, the bacteriological laboratories and their associated incubation rooms occupy a considerable proportion of the total area. The analytical laboratories are also equally prominent giving adequate working space for at least ten analysts. This area is further increased by a laboratory dealing with the control of factory operations, thereby keeping the routine control work separate from the more advanced analytical work on raw materials and finished products. In the same block are built the associated packaging inspection work shops, offices and tasting room.

The laboratories have been placed as near as possible to the centre of the three storey factory and are located in part of the central or mezzanine floor. This floor is 8 ft. high, and covers the same area as the manufacturing (upper floor) and filling and processing (lower floor) departments. These departments form half the complete factory block, the remainder being composed of storage and warehouse departments. The latter departments are therefore equally easily accessible from the laboratories.

The positioning of the laboratories gave immediate difficulties with regard to lighting. The laboratories are built along an outside glass wall of the factory building and thus adequate daylight is available. The daylight is controlled by venetian blinds which have been fitted to all windows. Since the factory operates at night, however, equally satisfactory electric lighting is required. This is obtained by generous use of fluorescent units which are fitted close to the ceiling (to make maximum use of the 8 ft. ceiling height) and are highly dispersed by perspex covers, to avoid glare. The routine laboratories and tasting rooms are fitted with specially colour-matched fluorescent tubes, to enable accurate assessment of product colour to be made.

The various areas are separated by de-mountable partitions which are fitted from floor to ceiling. These have a hard white plastic surface which is completely impervious, easily cleaned, and therefore particularly suitable for the bacteriological working areas.

The floors are covered with sheet polyvinylchloride (P.V.C.) which was laid prior to the building of the partitions. It is therefore possible for areas to be altered easily, and rooms relocated, without any difficulty being encountered with regard to floor covering. Benches have been similarly built on block principles throughout the laboratories, and the services (including lighting) provided at regular intervals so as to allow maximum possible freedom for re-location.

Polythene has been used for all sink drainage, and has also been used to pipe a supply of distilled water to all benches. The latter pure water is obtained from a de-ionizing unit located on the roof of the building. A reservoir of 50 gallons is provided in a polythene lined tank, the level being automatically controlled.

All areas are ventilated by ducted air the temperature of which is thermostatically controlled.

Colour has been carefully controlled throughout the whole of the factory. In the laboratories most of the walls are formed by plastic partitions which are white, but colour relief has been obtained by colouring other walls blue, and also from grey floors, mahogany work benches, blue formica bench surfaces and some primrose yellow tiling.

The various laboratories have features of special interest and are best considered separately.

Product Control Laboratory

The most important feature of the product control laboratory is the speed with which samples taken in the factory may be tested. High speed is obtained by passing the samples from various points through the manufacturing areas to the laboratory by Lamson tubes. These tubes transmit, by air circulation, carriers containing up to 400 ml. samples of product. The carriers are of special design and are fitted with an adjustable coding device which gives details of the type of sample, batch number, etc. The tests carried out in the control laboratory are of a routine nature, such as salt and acidity titrations, refractive indices, and moisture contents. Many hundreds of acidity titrations are carried out regularly, and constant accuracy is achieved by the use of an automatic titration instrument, based on the pH meter. A bench with special lighting is provided for normal titration operations. Moisture contents are carried out by drying, using cheap dispensible dishes to avoid unnecessary quantities of difficult dish washing; at the same time a large sink and electrically heated drying cupboard are provided in this laboratory and in other laboratories to assist with washing-up operations.

Analytical Laboratories

The functions of the analytical laboratories are:

(*a*) to analyse as far as required all ingredient materials, and compare them with laid down Heinz specifications;

(*b*) to carry out regular analysis of finished products;

(*c*) to advise production departments regarding their special problems. Such work ranges from testing scrubbing brushes used for cleaning purposes to complex analyses of metal alloys, plastics, etc.

The analytical laboratory has been specially equipped to handle food materials. A floor standing combined Kjeldhal distillation and digestion unit is used, the fumes from which are separately trunked to atmosphere through polythene ducts to the roof. An electrically heated Soxhlet apparatus is also provided in addition to normal ovens, water baths and furnaces. There is also a fully equipped glass blowing bench. Two smaller rooms are accessible from the main analytical laboratory. One is the balance room which is fitted with an insulated slate-topped bench. Fully automatic balances only are used, even for routine work, as these are found to withstand heavy usage and retain full accuracy better than the conventional balance and weights. The second smaller room is equipped as a laboratory, and is set aside for more delicate work, particularly work involving

A general view of the bacteriological laboratory.

instruments such as the polarimeter, refractometer, microscope and spectrophotometer, which are installed there.

The Bacteriological Laboratory

One of the main concerns of the bacteriological laboratory is the sterility check on finished products, and the arrangement of the various rooms permits this function to be carried out easily and quickly. The laboratory has adjoining it a media preparation room, incubation rooms, sterile plating-out room and photographic dark room. The temperature of the three incubation rooms is automatically controlled at 25°, 37° and 55°C., the walls, doors and ceiling being heavily heat-insulated. Samples of all production are incubated in these rooms and after a set period are passed via hypochlorite dip to the plating out room. Two-way cupboards form the wall between the plating out room and the media preparation area, and the necessary transfer of culture media is arranged through the cupboards. To prevent, as far as possible, aerial contamination the plating-out room is dust-proofed and a slight positive pressure is maintained with filtered air. The photographic dark room is used for the processing of photo-micrographs. Photomicrography is used over a wide field including normal bacteriological work, can-seam examination, ingredients examination, metallurgical work, etc., these forming only part of the wide field covered by the bacteriological department in addition to its basic function.

To summarize we may say that the laboratories have been designed and equipped in such a way that the most accurate and up-to-date equipment and techniques can be used in areas which are laid out in such a way as to allow maximum efficiency and adaptability. At the same time all possible consideration has been given to lighting, ventilation, decoration and other factors in order to give the best possible working conditions.

The equipment of the whole of these laboratories was undertaken by W. E. Marson & Co. Ltd.

A Royal Election

Under the Statute of the Royal Society which provides for the election of persons who either have rendered conspicuous service to the cause of science or are such that their election would be of signal benefit to the Society, His Majesty King Gustaf VI Adolf of Sweden has been elected a Fellow of the Royal Society.

Association of Public Analysts

At the Annual General Meeting of the Association of Public Analysts held on June 11, 1959, the following were elected as Officers of the Association for the ensuing year:

President, Mr. J. G. Sherratt; *Vice-President*, Dr. J. H. Hamence; *Past Presidents*, Mr. E. Voelcker, Mr. F. C. Bullock, Mr. H. E. Monk; *Hon. Treasurer*, Mr. R. C. Spalding; Hon. Editor, Dr. E. C. Wood; *Hon. Secretary*, Mr. F. A. Lyne; *Asst. Hon. Secretary*, Mr. D. F. H. Button.

LABORATORY EQUIPMENT & APPLIANCES

DCL 'M' Metering Pumps

The DCL 'M' range of metering pumps has been adapted for automatic control. Developed primarily for the accurate metering of small quantities of fluids, the new range can now be operated with pH, temperature, flow and other controls.

These automatically-controlled DCL 'M' pumps are responsive to pneumatic signals of from 3 to 15 p.s.i.g., adjustment of the plunger stroke being carried out by a small, compact bellows motor operating through a mechanism designed to reduce 'hunting'. Available with different plunger sizes giving a range of capacities from 0-750 cc./hour to 0-37·3 litres/hour, the pumps are suitable for working pressures up to a maximum of 2,400 p.s.i.g., the maximum pressure depending upon capacity. The output from each pump is proportional to the air pressure, the control gear being designed to give maximum capacity at either 3 or 15 p.s.i.g.

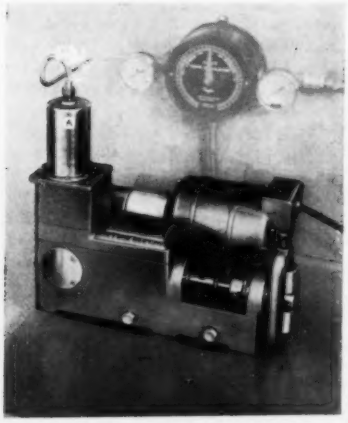
DCL metering pumps are of proved durability and ease of maintenance. Pumps heads are interchangeable, the head in each case being easily removed and replaced by one of different capacity. Power is provided by a 1/15 h.p. totally enclosed induction motor. The suction and delivery valves, both of which are duplicated, are of 12 per cent chromium non-corrodible steel balls specially lapped to ensure high volumetric efficiency. Valve bodies, seats and the valves themselves can be readily removed by merely slackening one set screw, to facilitate inspection and maintenance.

As with other types of DCL 'M' pumps, hydraulically operated diaphragm heads are available for applications where it is desirable to avoid having glands in contact with the liquid being pumped. This is especially useful in cases where noxious fluids are being handled or when sterile conditions are required.

Automatically controlled DCL 'M' metering pumps are an addition to the standard ranges of DCL equipment and are designed for all types of plant and laboratory applications. For special applications standard pumps and motors can be modified to suit individual requirements.

The present price of the standard 'M' plunger pump is £95 ex works, the 'M' pump with stainless diaphragm head being £130. When fitted with the bellows unit for automatic control, the prices are £145 and £180 respectively, normal delivery being ex stock.

The illustration shows the method of coupling the pump bellows control motor to a typical pneumatic temperature regulator from which it receives the varying air pressure signals.
(Manufacturers: The Distillers Co., Ltd., Great Burgh, Epsom, Surrey.)



The illustration shows the method of coupling the pump bellows control motor to a typical pneumatic temperature regulator from which it receives the varying air pressure signals.

The Ophitron

A compact microwave generator embodying a new focusing principle has been developed at the G.E.C. Research Laboratories for the Admiralty under the programme for Co-ordination of Valve Research and Development.

The valve is an electrostatically-focused backward-wave oscillator which has been named the 'Ophitron'.

The most striking advantages of the new oscillator are its small size (6 in. long x ¼ in. diameter) and low weight (7 oz.), and in addition the 'Ophitron' system has been designed to be simple to construct and to operate. A single stamped-out periodic structure and two flat focusing plates form the propagating path for the r.f. wave, and set up the periodic electrostatic-field which focuses the electron beam. The system has the fundamental advantage that the crests of the undulating electron beam are brought into the region of maximum r.f. field. This feature gives good coupling between beam and wave, and leads to greater bandwidth than is obtained with the equivalent magnetically focused backward-wave oscillator.

The electrostatically focused backward wave oscillator.

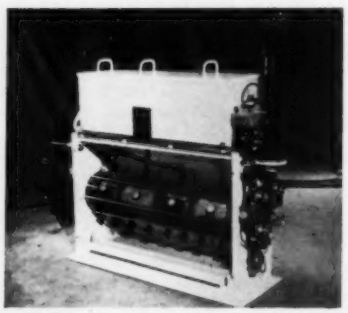
The present Ophitron tunes electronically over at least a 40 per cent band in the 10,000 Mc/s region. A range of such valves is envisaged, covering most important centimetre wavelength bands. It is expected that the noise performance will be better than that of magnetically focused backward-wave oscillators due to the ion drainage from the electron beam inherent in the focusing method.
(Manufacturers: M.O. Valve Co., Ltd., Hammersmith, London, W.6.)

The Stonwata A300 Deioniser

The A300 deioniser fills the gap between laboratory size and full production scale units and provides at the turn of a valve the immediate choice of 'conductivity' or 'distilled' water in quantity.

It is essentially two deioniser units in series: the first a multi-bed type of novel design which removes the bulk of the ions and yields water of 'distilled' quality or better; the second, fed from the first, is a conventional monobed cartridge, yielding water of 'conductivity' quality or better.

The multi-bed unit is regenerated *in situ*. Polythene tanks to contain the acid and alkali regenerants are provided on top of the apparatus. Concentrated acid and alkali are placed in these tanks and are diluted using deionised water fed



The Stonwata A300 deioniser.

through P.V.C. pipes built into the apparatus for this purpose.

The regenerants are fed in a vertical direction into the cells via the ebonite stopcocks. They then flow slowly downwards through the resins and out into the polythene drip tray provided. The micro-porous plastic filter discs are impervious to the regenerants owing to the low pressure head so that the cells are thereby effectively isolated during regeneration. The regeneration process takes approximately 50-60 min. but only 5 min. of operator time is required at the start and at the finish of the process. The cost of the acid and alkali regenerants is virtually the only cost.

To avoid corrosion by spillage of the regenerants, all the metalware is epoxy-coated, or chromium plated.

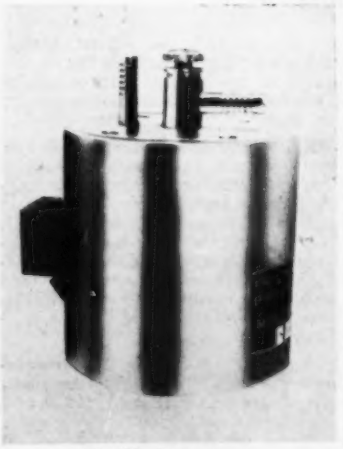
On exhaustion the cartridge must be returned for regeneration.

(Manufacturers: Loughborough Glass Co., Ltd., Loughborough, Leics.)

Pump for Research Laboratories

This new pump is basically a vibrator type of miniature size and so designed that it can be set to give the performance desired by the user. It is adaptable to a wide variety of technical operations in many industries, both as a component built into equipment and in research laboratories. It can also be used in medical and dental equipment.

The pump can be supplied in either a metal or bakelite cylindrical case and is particularly compact. Overall dimensions of the metal case model is 2½ in. long by 2⅜ in. diameter, weighing 24 oz. This model is particularly designed for inclusion in scientific or engineering equipment. The bakelite cased model is 3⅛ in. long, 3⅛ in. diameter and weighs 30 oz. This type is principally used for bench work.



The Singleton pump.

There is a wide range available to give positive or negative pressure in closed or open circuit. They are suitable for liquids or gases, including corrosive gases. They can be supplied for A.C. voltages from 24 volts upwards. Inlet and outlet connections and mountings can be adapted to requirements.

As the manufacturers produce these pumps to the individual requirements of each customer upon information supplied as to the performance required, it is difficult to give a general technical specification.

(Manufacturers: Singleton Bros. (Instruments) Ltd., Effingham, Surrey.)

Mixing Equipment

Two pieces of laboratory equipment, said to be new to this country, are now available. One is a micro mixer mill for extremely rapid disintegration and mixing of minute quantities of substances to ultra-fine particle size. The grinding and mixing is carried out in stoppered vials of 2 or 5 c.c. capacity, made of plastic, stainless steel, hardened tool steel or agate. The size of the vials and the fact that they are completely closed during operation ensures that even the smallest quantities can be ground and mixed without any losses and there is no risk of contamination. The large choice of vials of different materials is of particular value where even microscopically small traces of abraded contaminants from the grinding tools have to be considered.

The grinding speed is very fast: materials can be pulverized to less than 200 mesh in two to three minutes.

The mill can be used in many branches of science and industry. Apart from such specialized fields as electron microscopy, infra-red spectroscopy, x-ray diffraction studies, powder metallurgy, the mill is an extremely useful analytical and research tool in chemical and medical laboratories, with applications in biochemistry, agricultural and horticultural research, food investigation, etc.

The price has been set so low that the unit is brought within the reach of most laboratories, the simplest outfit costing only £37.

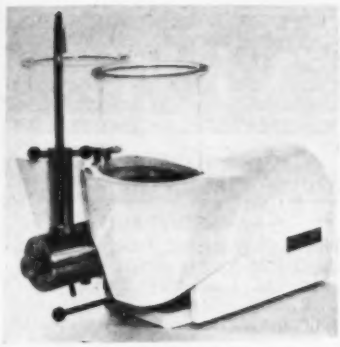
The other equipment is a sample mixer mill which basically serves the same purpose, but is capable of coping with very much larger quantities.

(Available from: Glen Creston Ltd., 41 Church Road, Stanmore, Middlesex.)

New Ultrasonic Homogenizer

A small versatile homogenizer at a reasonable price, known as the Minisonic Model 3, is described as three machines in one:

1. *A laboratory homogenizer* with which small quantities of emulsions and dispersions can be quickly made. Up to 3 litres can be treated at one filling without external premixing.



The Minisonic Model 3 laboratory and small batch homogenizer.

2. *A small batch homogenizer* for small batches of emulsions as required in the cosmetic industry or in hospital dispensaries.

3. *A pilot plant homogenizer* with which an emulsification process can be worked out on a small scale and immediately transferred to production using the Rapisonic homogenizer.

The principle upon which this model works is that of the liquid whistle covered by British Patent No. 695491 (and many foreign patents). It has been established as the most efficient method of utilizing cavitational forces in liquids and is fully described in many standard text-books.

The ingenious arrangement of this model permits four distinct methods of use:

(*a*) Gradual emulsification, which is particularly useful when the proportion of disperse phase approaches the theoretical limit or when there is danger of reversal in the emulsion system.

(*b*) Instantaneous emulsification, which will result in a continuous production process when using the Rapisonic.

(*c*) One pass homogenization for sonification of premixes.

(*d*) Multiple transfer for emulsions and organic dispersions which yield better results when they are sonified more than once.

During development work no limitations with regard to hot liquids have been met and a novel feature enables a very wide range of viscosities to be processed with equal efficiency. It is constructed so that all parts in contact with the liquid to be sonified are made in 18/8 stainless steel and corrosion resistant materials. All surfaces are polished, which greatly assists in cleaning; if necessary, the whole unit can be quickly dismantled. Built-in glass vessels and a three-way finger-tip control valve make for ease of operation and the Minisonic is supplied ready for plugging into a 15 amp. 3-pin socket.

(Manufacturers: Ultrasonics Ltd., Westgate, Otley, Yorks.)

NEWS

Manufacturers' News

Appointment of Distributors

The recently formed Analytical Instruments Department of Southern Instruments Ltd., Camberley, have appointed A. R. Bolton & Co., of Edinburgh, as their sole distributors for Scotland.

Formica Beautyboard

Formica Ltd. have just completed a £500,000 extension to their existing Tyneside factory for the production of Beautyboard—a combination of plastic decorative surface, a specially constructed laminated wood core and a plastic counter veneer. This new product will be available in the entire standard Formica range of colours and patterns in a bonded, composite board.

It will be particularly suitable for large area uses and will no doubt find some applications in the laboratory.

Change of Address

All goods and correspondence for Elga Products Ltd. and Deionisation (Elga) Ltd., should now be addressed to their new factory at Lane End, Bucks. (Phone: Lane End 396).

The Durgapur Steelworks Project

Early in 1958, Baird & Tatlock (London) Ltd. were appointed Consultants to the Indian Steelworks Construction Co. Ltd. (ISCON) regarding the laboratories for the £100 million Durgapur Steelworks; these comprise a coke oven laboratory, a blast furnace laboratory, a steel plant laboratory, a coal washing laboratory and a water testing laboratory. As a result of this appointment, Baird & Tatlock (London) Ltd.:

1. Prepared complete lists of apparatus and equipment to be supplied to the various laboratories.
2. Prepared drawings of the laboratory installations and fittings and supplied details of the standard B.T.L. wooden fittings required; the majority of these will be constructed in India.
3. Drew up a list of chemicals that will be required for the Steelworks.
4. Advised ISCON on the various aspects of the analysis of steel and its by-products; in particular, advice was given on the installation of modern automatic recording apparatus for gas analysis and steel analysis.

Baird & Tatlock (London) Ltd. have been appointed contractors for the supply of all the laboratory apparatus, chemicals and equipment for the laboratories of the Durgapur Steelworks; this will be handled in conjunction with their agents in India, Toshniwal Brothers (Private) Ltd.

The laboratories will use the most modern techniques of laboratory analysis available. In particular, the installation of direct-reading polychromators for steel analysis will avoid the more usual wet testing methods and enable analyses to be carried out in one to two minutes instead of over half an hour, thus greatly contributing to the efficiency of the Steelworks.

The Chief Metallurgist of the Durgapur Steelworks, Dr. G. P. Chatterjee, recently spent two months in England. During this time the Company were able to arrange for him to visit large steelworks in this country and a number of research laboratories. They were also able to show him some of the equipment that will be installed at Durgapur, and to discuss with him in greater detail the design and operation of the laboratories.

The new Lane End factory of Elga Products Ltd.

Change of Name

Davis & Thompson Ltd. of Watford By-Pass, Watford, Herts., have now changed their name to Datum Metal Products Ltd.

Personal Notes

Mr. T. P. Drewitt

Mr. T. P. Drewitt formerly, on the staff of J. A. Jackson (London) Ltd., for over 30 years is now in business as a supplier of laboratory, medical, surgical glassware at 378 City Road, London, E.C.1. *Tel.* Terminus 5776.

CATALOGUES, BROCHURES & LEAFLETS

Received from Manufacturers

Heating Equipment.—The Stabilag Co. Ltd., of Mark Road, Hemel Hempstead, Herts., have recently revised all their technical literature which is now available on request. The laboratory heating equipment leaflet covers laboratory heating mantles, Kjeldahl and Soxhlet extraction apparatus, pipe and column heaters, heating pads, mats, tapes and panels, funnel jackets and beakers, high-temperature heating mantles, pilot plant, controllers, etc.

B.D.H. Leaflets.—New entries in the catalogue of British Drug Houses Ltd. of Poole, Dorset, are listed in the June 1959 issue of their monthly leaflet. These are as follows: *Organic and Inorganic Chemicals.*—4-methyl-nioxime; methyl thymol blue complexone. *Microscopical Stains.*—Vital red.

Marconi Instrumentation.—The June 1959 issue of Marconi Instrumentation (Vol. 7, No. 2) published by Marconi Instruments Ltd., St. Albans, Herts., contains articles on cathode ray oscilloscopes, a new general purpose oscilloscope, a circuit magnification meter and test gear.

Heat-Treatment Journal.—Modern heat-treatment equipment, and mains frequency crucible type induction melting furnaces are the subjects of the main articles in the current issue (Vol. VII, No. 51) of the Wild-Barfield Heat-Treatment Journal published by Wild-Barfield Electric Furnaces Ltd. (and associated companies) of Elecfurn Works, Otterspool Way, Watford By-Pass, Watford, Herts.

Recording Infra-red Spectrophotometer.—A new brochure fully describing and illustrating the Hilger H 800 recording infra-red spectrophotometer has been published by Hilger & Watts Ltd. 98 St. Pancras Way, London, N.W.1.

He's out to prove something

Most of us, in our formative years had decided views that this gentleman never really deserved laurel leaves. However, he reached some very sound conclusions. We have a few theories also, about the installation and equipping of laboratories. If this is one of *your* problems, we may be able to help in getting that quart into a pint pot

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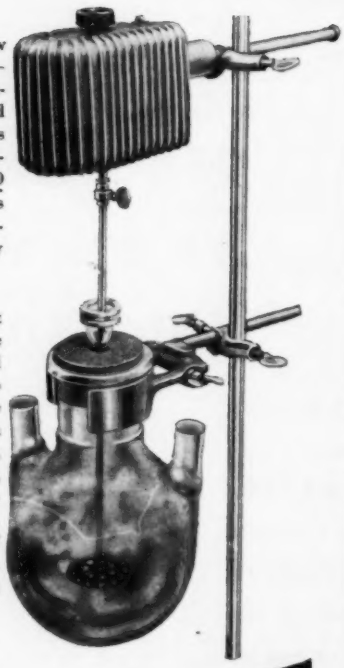

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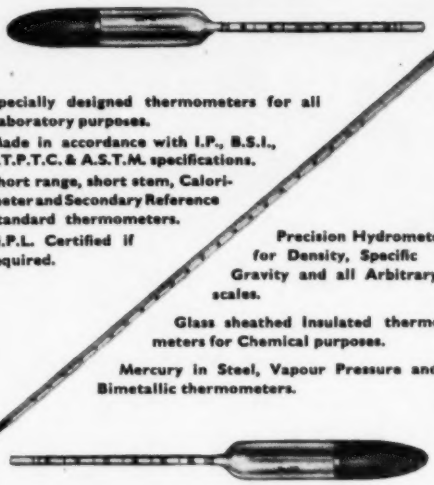

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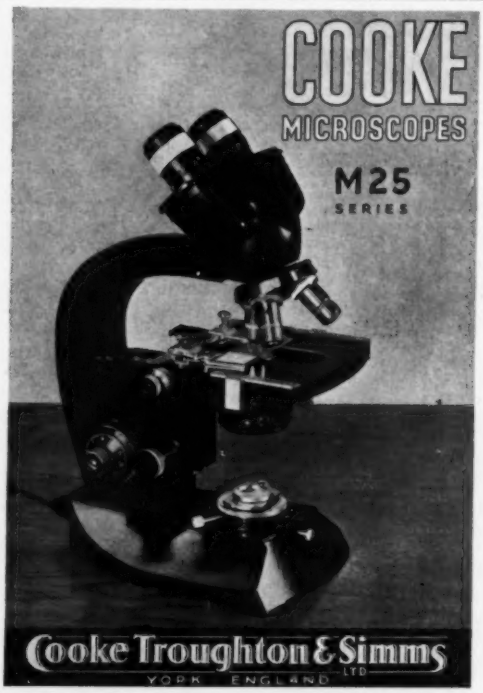

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Mill illustrated is an extremely useful unit for carrying out several jobs at the same time on one or more materials of varying quantity or time of processing.

Arranged with three separate motors and with three pairs of 2½" diameter white rubber bonded rolls in three tiers. Usable with all three in operation or one or two separately. Each tier will carry two 1-gallon or three ½-gallon or four 2-pint or five 1-pint pots and combinations of these can be operated at the same time

Mill shown is only one of many types available from miniature 1-pint model for processing small quantities of expensive materials to larger 5-gallon model with tilting cradle in which the pot is securely held, charged, operated and discharged without having to lift this heavy pot from cradle.

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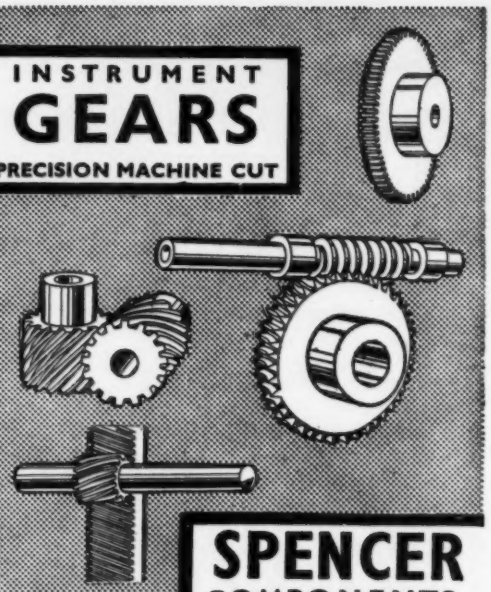

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D. Colver Nutting, M.B.E., A.R.C.S., B.Sc.

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The author has given a concise treatment of the subject in the five articles now available as a bound set of reprints. The underlying principles are reviewed and the derivation of the pH scale explained. Methods of measurement and control are detailed, and the application to a variety of typical continuous processes is outlined. The problem of close control is dealt with by the Author.

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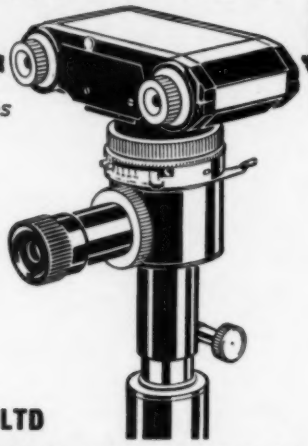

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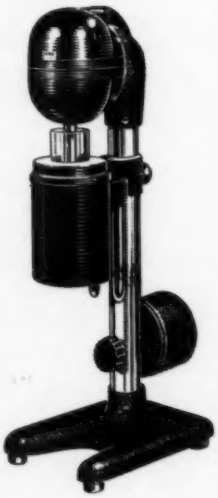
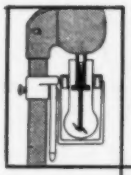
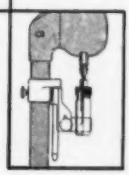




Cross-section showing how the 100 c.c. beaker is accommodated in the plastic container, providing space for coolant



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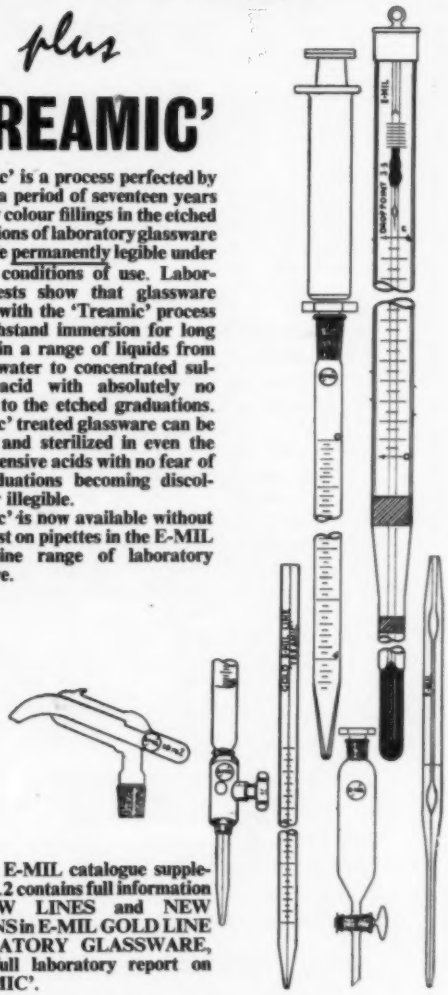

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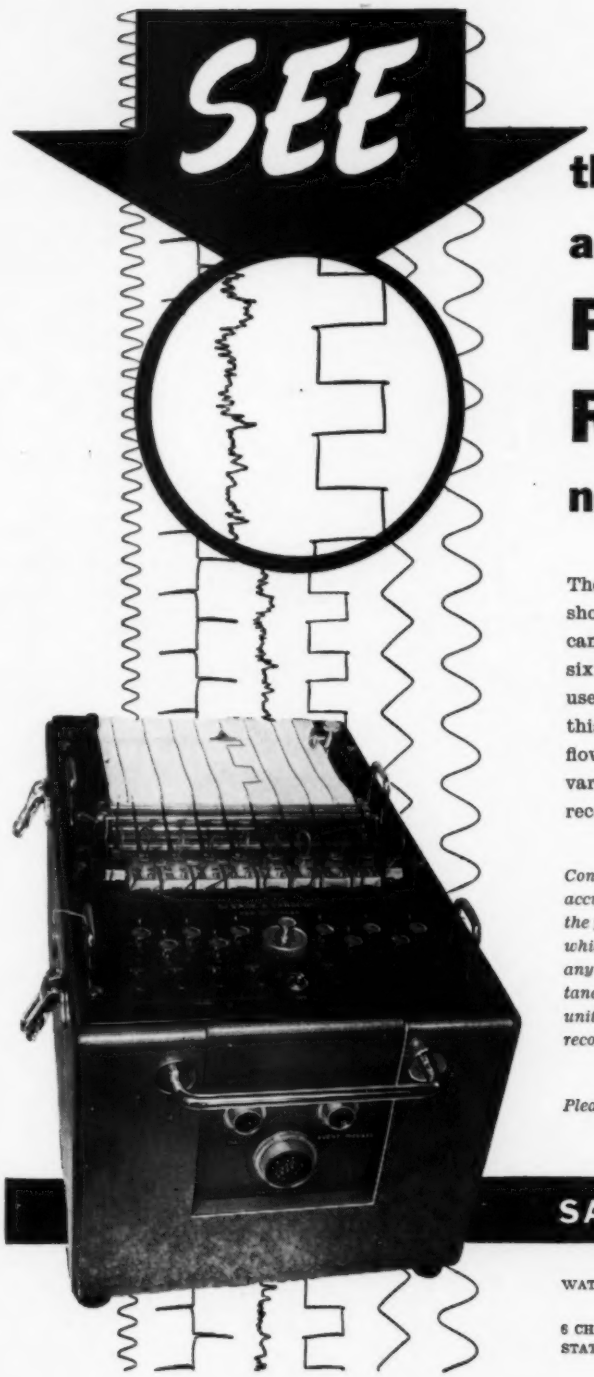

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